EPA United States Environmental Protection Agency

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News Releases from Headquarters

EPA Settles with DuPont over Violations of Federal Pesticide Laws that Led to Widespread Tree Deaths and Damage/ DuPont to pay $1,853,000 penalty to resolve alleged violations of pesticide reporting and distribution laws

Release Date: 09/15/2014
Contact Information: Jennifer Colaizzi, Colaizzi.jennifer@epa.gov, 202-564-5677

WASHINGTON – The U.S. Environmental Protection Agency (EPA) today announced a settlement with the E.I. du Pont de Nemours and Company (DuPont) for alleged violations of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). DuPont will pay a $1,853,000 penalty to resolve allegations that the company failed to submit reports to EPA about potential adverse effects of an herbicide product called Imprelis, and sold it with labeling that did not ensure its safe use. When customers applied the misbranded Imprelis product, it led to widespread death and damage to trees.

"EPA's ability to protect the public from dangerous pesticides depends on companies complying with the legal obligation to disclose information on the harmful effects of chemicals," said Cynthia Giles, EPA Assistant Administrator for Enforcement and Compliance Assurance. "This case sends the message that illegally withholding required information will be treated as a very serious violation."

As part of the registration process for a pesticide or herbicide, FIFRA requires companies to submit to EPA reports on a product's potential adverse impacts on plants or animals that it is not intended to control. During the registration process and after registration was approved for Imprelis, an herbicide product intended to control weeds like dandelions, clover, thistle, plantains and ground ivy, DuPont failed to submit 18 reports.

As a result, Imprelis – as it was registered and labeled – did not adequately protect against damage to certain tree species. DuPont made 320 shipments of Imprelis to distributors in 2010 and 2011. This failure to submit reports and the sale or distribution of a misbranded pesticide or herbicide are violations of FIFRA.

DuPont has submitted over 7,000 reports to EPA of damage or death of trees – primarily Norway spruce and white pine – related to the application of Imprelis. Test data from DuPont confirmed certain coniferous trees, including Norway spruce and balsam fir, as susceptible to being damaged or killed by the application of Imprelis. There is also evidence that non-coniferous trees such as maple, honey locusts, lilacs, sycamores, and alders are susceptible to damage from Imprelis.

Starting in June 2011, EPA began receiving complaints from state pesticide agencies regarding damage to trees related to the use of Imprelis when it was applied to control weeds. Cases of tree damage and death from Imprelis were widespread in the Midwest, especially Indiana, Illinois, Michigan, Minnesota, Ohio and Wisconsin. Indiana investigated more than 400 cases of tree damage related to Imprelis in 2011.

In August 2011, EPA ordered DuPont to stop selling and distributing Imprelis without prior approval from EPA. In September 2011, the registration for Imprelis was amended to prohibit the sale, distribution or marketing of Imprelis. The product registration for Imprelis expired on September 8th, 2014, and DuPont is no longer selling the product.

Imprelis was distributed and sold in 1 gallon, 2.5 gallon and 4.5 ounce containers, primarily to pest control professionals servicing the lawn, golf, turf and weed control sectors.

Imprelis was registered with EPA in 2010, and was marketed by DuPont for lawn and turf applications on residential and commercial lawns, golf courses, sod farms, schools, parks, and athletic fields.

The settlement, a consent agreement and final order, will be filed at EPA's regional office in Philadelphia, and DuPont must submit payment of the penalty to the U.S. Department of Treasury within 30 days.

For more information about this settlement, click here: http://www2.epa.gov/enforcement/ei-du-pont-de-nemours-and-company-settlement

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The Task Force on Systemic Pesticides

EMBARGOED TO: 00.01 CET 24 JUNE 2014

PRESS RELEASE

NEW FOUR-YEAR SCIENTIFIC ANALYSIS: SYSTEMIC PESTICIDES POSE GLOBAL THREAT TO BIODIVERSITY AND ECOSYSTEM SERVICES

The conclusions of a new meta-analysis of the systemic pesticides neonicotinoids and fipronil (neonics) confirm that they are causing significant damage to a wide range of beneficial invertebrate species and are a key factor in the decline of bees.

Concern about the impact of systemic pesticides on a variety of beneficial species has been growing for the last 20 years but the science has not been considered conclusive until now.

Undertaking a full analysis of all the available literature (800 peer reviewed reports) the *Task Force on Systemic Pesticides* – a group of global, independent scientists - has found that there is clear evidence of harm sufficient to trigger regulatory action.

The analysis, known as the *Worldwide Integrated Assessment* (WIA), to be published* in the peer reviewed Journal *Environment Science and Pollution Research*, finds that neonics pose a serious risk of harm to honeybees and other pollinators such as butterflies and to a wide range of other invertebrates such as earthworms and vertebrates such as birds.

Neonics are a nerve poison and the effects of exposure range from instant and lethal to chronic. Even long term exposure at low (non-lethal) levels can be harmful. Chronic damage can include: impaired sense of smell or memory; reduced fecundity; altered feeding behaviour and reduced food intake including reduced foraging in bees; altered tunneling behaviour in earthworms; difficulty in flight and increased susceptibility to disease.

One of the lead authors of the WIA, Dr Jean-Marc Bonmatin of The National Centre for Scientific Research in France said: "The evidence is very clear. We are witnessing a threat to the productivity of our natural and farmed environment equivalent to that posed by organophosphates or DDT. Far from protecting food production the use of neonics is threatening the very infrastructure which enables it, imperilling the pollinators, habitat engineers and natural pest controllers at the heart of a functioning ecosystem."

The analysis found that the most affected groups of species were terrestrial invertebrates such as earthworms which are exposed at high levels via soil and plants, medium levels via surface water and leaching from plants and low levels via air (dusts). Both individuals and populations can be adversely affected at even low levels and by acute (ongoing) exposure. This makes them highly vulnerable to the levels of neonics associated with agricultural use.

The next most affected group is insect pollinators such as bees and butterflies which are exposed to high contamination through air and plants and medium exposure levels through water. Both individuals and populations can be adversely affected by low or acute exposure making them highly vulnerable.

Then aquatic invertebrates such as freshwater snails and water fleas which are vulnerable to low and acute exposure and can be affected at the individual, population and community levels.

While vertebrate animals are generally less susceptible, bird populations are at risk from eating crop seeds treated with systemic insecticides, and reptile numbers have declined due to depletion of their insect prey. Microbes were found to be affected after high levels of or prolonged exposure. Samples taken in water from around the world have been found to exceed ecotoxicological limits on a regular basis.

In addition to contaminating non-target species through direct exposure (e.g. insects consuming nectar from treated plants), the chemicals are also found in varying concentrations outside intentionally treated areas. The water solubility of neonics mean that they leach and run-off easily and have been found to contaminate much wider areas leading to both chronic and acute exposure of organisms, including in riparian zones, estuarine and coastal marine systems.

They have become the most widely used group of insecticides globally, with a global market share now estimated at around 40% and sales of over US $2.63 billion in 2011. They are also commonly used in domestic treatments to prevent fleas in cats and dogs and termites in wood structures..

Chair of the Task Force, Maarten Bijleveld van Lexmond said: "The findings of the WIA are gravely worrying. We can now clearly see that neonics and fipronil pose a risk to ecosystem functioning and services which go far beyond concerns around one species and which really must warrant government and regulatory attention."

Honey bees have been at the forefront of concern about neonics and fipronil to date and limited actions have been taken, for example by the EU Commission, but manufacturers of these neurotoxicants have refuted any claims of harm. In reviewing all the available literature rather than simply comparing one report with another, the WIA has found that field-realistic concentrations of neonics adversely affect individual navigation, learning, food collection, longevity, resistance to disease and fecundity of bees. For bumblebees, irrefutable colony-level effects have been found, with exposed colonies growing more slowly and producing significantly fewer queens.

The authors strongly suggest that regulatory agencies apply more precautionary principles and further tighten regulations on neonicotinoids and fipronil and start planning for a global phase-out or at least start formulating plans for a strong reduction of the global scale of use.

ENDS

NOTES

*** The full WIA will be published in the Springer Journal within the next few weeks. Date to be confirmed by the Journal**

Systemic Pesticides

Unlike other pesticides, which remain on the surface of the treated foliage, systemic pesticides are taken up by the plant and transported to all the tissues (leaves, flowers, roots and stems, as well as pollen and nectar). They are increasingly used as a prophylactic to prevent pests rather than to treat a problem once it has occurred.

The metabolites of neonics and fipronil (the compounds which they break down into) are often as or more toxic than the active ingredients to non-target organisms. Both parent compound and some of their metabolites are able to persist and environmental concentrations can build up, particularly in soil, over months or years. This increases their toxicity effects and makes them more damaging to non-target species.

Task Force On Systemic Pesticides

The Task Force on Systemic Pesticides is the response of the scientific community to concern around the impact of systemic pesticides on biodiversity and ecosystems. Its intention is to provide the definitive view of science to inform more rapid and improved decision-making.

It advises two IUCN Commissions, the *Commission on Ecosystem Management* and the *Species Survival Commission.* Its work has been noted by the *Subsidiary Body on Scientific, Technical and Technological Advice* under the Convention on Biodiversity (CBD) and was brought to the attention of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services (IPBES) – on which four members of the Task Force serve - in the context of the fast-track thematic assessment of pollinators, pollination and food production.

Press Conferences releasing the findings will be held in Manila and Brussels on the 24th June, Ottawa on the 25th and Tokyo on the 26th.

Media Briefing notes available on request.

www.tfsp.info (live on 24th June 2014)

For further information please contact:

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Courthouse News Service

Monday, September 22, 2014 Last Update: 9:44 AM PT

Pesticide Label Challenge Thrown Out of Court

By REBEKAH KEARN

SAN FRANCISCO (CN) - Federal regulators that strung along environmentalists for eights years about plans to label inert pesticides ingredients cannot be sued, a federal judge ruled.

At issue here is an EPA requirement for pesticide manufacturers to list active, but not inert, ingredients, on their labels.

In 2006, the Center for Environmental Health and Californians for Pesticide Reform petitioned the EPA to require the labeling of 374 inert chemicals on pesticide bottles that "have been determined to be hazardous under other environmental laws and regulated as such by the EPA."

Though the group lost its court battle, a spokesperson with the EPA said relief may still be at hand because the agency "is considering reviewing the inert ingredients in the petition currently listed for use in pesticides to determine which ones are still used in pesticides."

It is possible that such analysis could prompt revisions to "the list of inert ingredients approved for use in pesticide products," the spokesperson added.

Once the EPA has criteria for prioritization, it can then select "top-candidate inert ingredients for further analysis and potential action to address those risks."

Carolyn Cox with the Center for Environmental Health voiced disappointment with the outcome.

"The agency had really been looking at good changes for progress on the issue and has now just backed off," she said in a telephone interview.

Center for Environmental Health had brought its lawsuit in 2009, after waiting three years for a response to its petition.

That action prompted the EPA to issue an advance notice of proposed rulemaking on the issue of disclosing inert ingredients, including nonhazardous chemicals. The EPA emphasized, however, that it was "not committing, and indeed legally cannot commit, to any particular outcome for rulemaking."

Though the group withdrew its complaint because of this development in 2010, it filed another suit this year because the EPA took no further action on the matter after four years.

The EPA had told the court that it was exploring different approaches to the issue and would not pursue making a rule that mandates disclosure of inert.

Though the environmental challengers noted that the EPA had taken eight years to fully respond to and deny their petition, U.S. District Judge William Orrick dismissed the case last week.

The EPA committed to nothing beyond issuing the advanced notice of proposed rulemaking, effectively concluding all action on the plaintiffs' petitions, the 6-page ruling states. [p. 5 lines 6-13]

"That the EPA has indicated that it is considering (but not committing to) action which arguably parallels part of what the plaintiffs requested in their original petition does not mean that the EPA has retroactively granted the portion of the plaintiffs' petition that the EPA denied in 2009," Orrick wrote (parentheses in original). "Plaintiffs are understandably frustrated that they may be no closer to fulfilling their goal eight years after petitioning the EPA to require that pesticide product labels list hazardous inert ingredients. But the EPA has unambiguously 'concluded' the 'matters' presented to it in plaintiffs' petitions, as required under the Administrative Procedures act, 5 U.S.C. §553(e), and I can offer the plaintiffs no relief. This matter is moot, a deficiency which cannot be cured by amendment."

Cox said the groups "have not had time to strategize about the next best approach," but said they "definitely have some next steps," including filing another petition.

"Because pesticides are something we are all exposed to every day, there are a lot of compelling reasons to know the ingredients," she said.

One such reason is giving doctors the information necessary to treat any patient who might be poisoned by a particular pesticide.

"This has been an important, controversial issue for decades, and we will keep working with [the EPA] until we can make some progress on it," Cox said. "We are definitely not going away."

Capital Press
The West's Ag Website

Air monitoring finds low pesticide levels in Calif.

Tim Hearden
Capital Press

Published:
September 24, 2014 9:08AM

Jing Tao, a scientist for the California Department of Pesticide Regulation, checks pesticide residue levels at an air monitoring station in Salinas, Calif. State air monitoring in three agricultural communities has found pesticide levels at far below those that would cause health concerns. COURTESY DEPARTMENT OF PESTICIDE REGULATION

Air monitoring in three California agricultural communities has found pesticide residue at levels far below those that would cause health concerns, a state agency has announced.

Capital Press

SACRAMENTO — For the third straight year, state air monitoring in three agricultural communities has found pesticide residues at well below levels that would cause a health concern.

California's Department of Pesticide Regulation has been monitoring air quality in Salinas, Ripon and Shafter, looking for particles from 32 pesticides and five pesticide breakdown products.

In 2013, nearly 93 percent of the 6,033 analyses that state scientists made resulted in no detectable concentrations, the agency announced on Sept. 23.

"We have found that a majority of the monitored pesticides were well below any levels that would need any further evaluation," DPR spokeswoman Charlotte Fadipe said. "We're very proud of our air monitoring network. We're the only regulatory agency that does something like this and it's something California decided to do just to give us some real data so we can know what's in the air."

No state or federal agency has set health standards for pesticides in air, but the DPR developed health screening levels to determine whether existing restrictions on pesticide applications adequately protect people.

Salinas, Ripon in San Joaquin County and Shafter in Kern County were chosen based on pesticide use on surrounding farmland and certain demographics, including the percentage of children, the elderly and farmworkers in the local population, the DPR has explained.

The scientists test for traces of methyl bromide and other major fumigants as well as other pesticides selected based on their potential health risks and the amount used, the department explained in a news release.

High readings prompted the DPR in February to limit growers' use of Telone, a powerful soil fumigant used for battling nematodes. In addition, the agency is preparing a series of proposals for chloropicrin, a broad-spectrum pesticide most prevalent in the

Salinas area, Fadipe said.

Measures for lowering chloropicrin residues in air could include different types of tarps, use of buffer zones and notification of neighbors, she said. Proposals should be out for public comment in five or six weeks, she said.

Of the pesticides and breakdown products monitored last year, 13 could not be detected at all and 10 were only detected at trace levels, the DPR's release stated. The pesticides detected the most often were chlorothalonil, chloropyrifos and methyl isothiocyanate, which were found at low levels about 30 percent of the time at all of the monitoring stations, according to the agency.

The state's testing comes as the phaseout of methyl bromide because of ozone depletion has led to intense research into alternatives in recent years. One alternative, methyl iodide, was cleared for use but pulled from the market several years ago amid criticism of the product from farmworkers and environmentalists.

Uses of Telone could decrease further if a new non-fumigant nematicide, fluensulfone, is approved for tree nuts and other crops. The product has just won approval from the U.S. Environmental Protection Agency but still must be registered in California.

Prevalence and impacts of genetically engineered feedstuffs on livestock populations[1]

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ABSTRACT: Globally, food-producing animals consume 70 to 90% of genetically engineered (GE) crop biomass. This review briefly summarizes the scientific literature on performance and health of animals consuming feed containing GE ingredients and composition of products derived from them. It also discusses the field experience of feeding GE feed sources to commercial livestock populations and summarizes the suppliers of GE and non-GE animal feed in global trade. Numerous experimental studies have consistently revealed that the performance and health of GE-fed animals are comparable with those fed isogenic non-GE crop lines. United States animal agriculture produces over 9 billion food-producing animals annually, and more than 95% of these animals consume feed containing GE ingredients. Data on livestock productivity and health were collated from publicly available sources from 1983, before the introduction of GE crops in 1996, and subsequently through 2011, a period with high levels of predominately GE animal feed. These field data sets, representing over 100 billion animals following the introduction of GE crops, did not reveal unfavorable or perturbed trends in livestock health and productivity. No study has revealed any differences in the nutritional profile of animal products derived from GE-fed animals. Because DNA and protein are normal components of the diet that are digested, there are no detectable or reliably quantifiable traces of GE components in milk, meat, and eggs following consumption of GE feed. Globally, countries that are cultivating GE corn and soy are the major livestock feed exporters. Asynchronous regulatory approvals (i.e., cultivation approvals of GE varieties in exporting countries occurring before food and feed approvals in importing countries) have resulted in trade disruptions. This is likely to be increasingly problematic in the future as there are a large number of "second generation" GE crops with altered output traits for improved livestock feed in the developmental and regulatory pipelines. Additionally, advanced techniques to affect targeted genome modifications are emerging, and it is not clear whether these will be encompassed by the current GE process-based trigger for regulatory oversight. There is a pressing need for international harmonization of both regulatory frameworks for GE crops and governance of advanced breeding techniques to prevent widespread disruptions in international trade of livestock feedstuffs in the future.

Key words: genetic engineering, genetically modified organisms, livestock feed, safety

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INTRODUCTION

The first genetically engineered (**GE**) feed crops were introduced in 1996. Their subsequent adoption has been swift. In 2013, GE varieties were planted on more than 95% of sugar beet, 93% of soy, and 90% of all cotton and corn acres in the United States (USDA National Agricultural Statistics Service, 2013). Global livestock populations constitute the largest consumers of GE feed crops. Independent studies have shown the compositional equivalence of the current generation of GE crops (Cheng et al., 2008; Garcia-Villalba et al., 2008; Herman and Price, 2013; Hollingworth et al., 2003), and no significant differences in feed digestibility, performance, or health have been observed in livestock that consume GE feed (Flachowsky et al., 2012). Similarly, it is not possible to detect differences in nutritional profiles of animal products after consumption of GE feed (Guertler et al., 2010; Tufarelli and Laudadio, 2013).

Despite these findings, some states have considered legislation that would require mandatory GE labeling

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of meat, milk, and eggs derived from animals that have eaten GE feed (CAST, 2014). Furthermore, some food companies are actively targeted by campaigns to promote products from animals that are fed non-GE diets. Given the widespread adoption of GE crops, the segment of animal agriculture that is currently feeding non-GE diets is relatively small. Approximately 0.8% of U.S. cropland and 0.5% of U.S. pasture were certified organic in 2011 (USDA National Agricultural Statistics Service, 2012), and only a portion of organic crops are used for animal feed.

Our objective was to briefly review the literature on livestock GE feeding studies and the composition of animal products derived from animals fed a GE diet. We gave special attention to health studies of animals, including an analysis of publicly available data on the health of commercial livestock populations since the introduction of GE crops in 1996. Also, we summarized the global usage and trade of GE feedstuffs along with the estimated size of GE-sensitive markets. Finally, we discussed issues regarding pipeline and regulation of GE crops with modified output traits, asynchronous regulatory approvals, and novel breeding technologies.

Livestock Feeding Studies with Genetically Engineered Feed

A total of 165 GE crop events in 19 plant species, including those used extensively in animal feed (alfalfa, canola, corn, cotton, soybean, and sugar beet), have been approved in the United States (James, 2013). Before approval, each new GE crop goes through a comprehensive risk assessment. The risk analysis of GE organisms is governed by internationally accepted guidelines developed by the Codex Alimentarius Commission (www.codexalimentarius.org). One leading principle is the concept of substantial equivalence, which stipulates that any new GE variety should be assessed for its safety by comparing it with an equivalent, conventionally bred variety that has an established history of safe use. Over the past 20 yr, the U.S. Food and Drug Administration found all of the 148 GE transformation events that they evaluated to be substantially equivalent to their conventional counterparts, as have Japanese regulators for 189 submissions (Herman and Price, 2013). By contrast, plant varieties developed through other processes of achieving genetic changes (e.g., radiation mutagenesis) go through no formal risk assessment before being placed on the market. There have been instances where plants bred using classical techniques have been unsuitable for human consumption. For example, the poison α-solanine, a glycoalkaloid, was unintentionally increased to unacceptable levels in certain varieties of potato through plant breeding resulting in certain cultivars being withdrawn from the U.S. and Swedish markets due to frequently exceeding the upper safe limit for total glycoalkaloid content (Petersson et al., 2013).

The difficulties associated with the safety and nutritional testing of whole foods/feed derived from GE crops, which contain thousands of bioactive substances, are well known (reviewed in Bartholomaeus et al., 2013). These include the fact that the quantity of the GE food that can be included in the diet of test animals is limited by the potential to generate nutritional imbalances and might not be high enough to detect adverse effects. Substantial differences in composition could be present without producing a recognizably meaningful difference between treatment groups fed whole foods. Many toxicologists concur that animal feeding trials of whole GE food have a low power to detect adverse effects and contribute little, if anything, to the safety assessment of whole foods (Kuiper et al., 2013). Far more sensitive analytical, bioinformatical, and specific toxicological methods exist to identify unintended effects resulting from plant breeding and provide more precise and quantifiable data for the safety evaluation of whole foods.

In 2013, the European Union (**EU**) Standing Committee on the Food Chain and Animal Health (Brussels, Belgium) adopted a regulation mandating a 90-d subchronic rodent feeding study (OECD, 1998) for every single GE transformation event. This is despite the fact that the European Food Safety Authority (2008; Parma, Italy) states that such testing is only warranted when driven by a specific hypothesis indicated by molecular, compositional, phenotypic, agronomic, or other analysis (e.g., metabolic pathway considerations) of the particular GE event. This mandate is seen by some as interference in the risk assessment of GE foods based on pseudoscience or political considerations (Kuiper et al., 2013). The United States and Australia/New Zealand explicitly do not require a 90-d subchronic rodent feeding study or actively discourage their conduct due to their negligible scientific value.

Studies in which GE crops are fed to target (food-producing) animals have focused less on GE risk assessment and more on evaluating the nutritional properties of the GE crop as well as resulting animal performance and health as compared to the results when fed an isogenic counterpart. Clear guidelines on experimental design for these types of studies have been developed (International Life Sciences Institute, 2003, 2007).

Multiple generations of food animals have been consuming 70 to 90% of harvested GE biomass (Flachowsky et al., 2012) for more than 15 yr. Several recent comprehensive reviews from various authors summarize the results of food-producing animal feeding studies with the current generation of GE crops (Deb et al., 2013; Flachowsky, 2013; Flachowsky et al., 2012; Tufarelli and Laudadio, 2013; Van Eenennaam, 2013). Studies have been conducted with a variety of food-producing animals including sheep, goats, pigs, chickens, quail, cattle, water buffalo, rabbits, and fish fed different GE crop varieties. The results have consistently revealed that the performance and health of GE-fed animals were comparable with those fed near-isogenic non-GE lines and commercial varieties. Many authors came to the same conclusion a decade ago (Aumaitre et al., 2002; Faust, 2002), suggesting that little contradictory data has emerged over the past 10 yr, despite the increased global prevalence of GE feed.

A number of long-term (of more than 90 d and up to 2 yr in duration) feeding trials and multigenerational studies conducted by public research laboratories using various animal models including pigs, cows, quail, and fish have also been reviewed (Ricroch, 2013; Ricroch et al., 2013; Snell et al., 2012). Significant among these studies are 2 thorough multigenerational studies that examined the long-term effects of feeding a GE corn variety (MON810, expressing the insecticidal Cry1Ab protein from *Bacillus thuringiensis* [**Bt**], one of the few GE corn varieties approved for cultivation in the EU) to food-producing animals, specifically, a German study in dairy cattle and an Irish study in pigs (Guertler et al., 2010, 2012; Steinke et al., 2010; Walsh et al., 2011, 2012 a, b, 2013; Buzoianu et al., 2012 a, b, c, d, 2013 a, b). The results from the multiple papers resulting from these 2 studies are summarized in Table 1. These studies were notable in that they included appropriate controls consuming isogenic non-GE lines of corn, and both comprehensively examined a range of phenotypes and indicators of growth and health and also used sophisticated techniques to look for the presence of recombinant DNA (**rDNA**) and Bt protein in the tissues and products derived from these GE-fed animals.

Results from these comprehensive studies revealed the compositional and nutritional noninferiority of GE corn to its isogenic control and an absence of long-term adverse effects from GE corn consumption. Organ pathology and function were similar between animals fed GE and non-GE corn, and there were no adverse effects of feeding GE corn on small intestinal morphology or the gut microbiota. Antibodies specific to the GE corn protein (Cry1Ab) were not detected in the blood, indicating the absence of an allergic-type immune response to the protein. Neither the *cry1Ab* gene nor the Cry1Ab protein was found in the blood, organs, or products of animals fed GE corn, indicating that neither the intact rDNA nor the intact recombinant protein migrated from the digestive system of the animal into other body tissues or edible animal products.

Even though these 2 comprehensive studies overwhelmingly revealed that a diet of Bt corn was not associated with long-term deleterious effects on the immune systems or animal performance, there were statistically significant differences in some of the parameters measured. Although the authors concluded that these differences were not of biological relevance, significant findings in any parameter in animal feeding studies have been interpreted by some as evidence of harm (Dona and Arvanitoyannis, 2009). Others have pointedly responded that statistical differences per se are not "adverse effects" and need to be considered in terms of their biological importance (Rickard, 2009). The European Food Safety Authority clarified the difference between statistical significance and biological relevance (European Food Safety Authority, 2011). In the absence of some predefined understanding of what changes might be of biological relevance, studies risk becoming "hypothesis-less fishing trips." Post hoc analysis of a large number of variables in a data set with a small sample size can lead to spurious conclusions because such studies "are fraught with differences that are not biologically significant between groups from simple variation and probability" (DeFrancesco, 2013).

The Federation of Animal Science Societies maintains an extensive bibliography of food-producing animal GE feeding studies (FASS 2014). Given the large number of 90-d subchronic rodent and food-producing animal GE feeding studies that currently exist in the literature, it is worth questioning the value of more animal feeding studies as part of a GE risk assessment for crops that are substantially equivalent to conventional comparators (Flachowsky, 2013). The rationale for conducting long-term feeding trials and multigenerational studies need to be explicitly stated, especially given that GE proteins are digested in the gut and no intact GE protein has been found in the bloodstream. Once compositional equivalence has been established for a GE crop, animal feeding studies add little to the safety assessment (Bartholomaeus et al., 2013).

There are less than 100 long-term (>90 d) and multigenerational target animal GE feeding studies in the peer-reviewed literature, which has prompted some to call for more of these types of feeding studies (DeFrancesco, 2013). Although such studies may seem intuitively appealing, they must result in novel useful data to justify the additional time, expense, and animal experimentation. Objective analyses of available data indicate that, for a wide range of substances, reproductive and developmental effects observed in long-term studies are not potentially more sensitive endpoints than those examined in 90-d rodent subchronic toxicity tests (European Food Safety Authority, 2008). There is no evidence that long-term and multigenerational feeding studies of the first generation of GE crops that have been conducted to date have uncovered adverse effects that were undetected by short-term rodent feeding studies (Snell et al., 2012). In the context of GE feed risk assessment, they argue that the decision to conduct long-term and

Table 1. Summary results of 2 comprehensive evaluations of target animal effects of long-term feeding of genetically engineered feed (Bt-MON810 corn) to dairy cattle and pigs[1]. Table adapted from Ricroch et al. (2013)

Study Design	Methods	Results	Conclusions	Reference
A. Dairy cattle study				
36 Simmental dairy cows (9 primiparous and 9 multiparous per treatment group) were assigned to 2 feeding groups and fed with diets based on whole-crop silage, kernels, and whole-crop cobs from GE corn (Bt-MON810) or its isogenic non-GE counterpart as main components. The 765-d study included 2 consecutive lactations.	Feed intake, milk production and composition, and body condition over 25 mo	There were no consistent effects of feeding GE corn or its isogenic control on milk composition or body condition. All changes fell within normal ranges.	Compositional and nutritional equivalence of GE corn to its isogenic control. No long-term effects.	Steinke et al. (2010)
	Gene expression pattern of markers for apoptosis, inflammation, and cell cycle from gastrointestinal tract and samples from liver	Statistical analysis of the examined gene expression pattern revealed no significant difference in the gene expression profile of cows fed transgenic or near-isogenic feed ration	Genetically engineered maize MON810 does not have any effect on major genes involved in apoptosis, inflammation, and cell cycle in the gastrointestinal tract and in the liver of dairy cows.	Guertler et al. (2012)
	Fate of *cry1Ab* DNA and recombinant protein	All blood, milk, and urine samples were free of recombinant DNA and protein. The *cry1Ab* gene was not detected in any fecal samples; however, fragments of the Cry1Ab protein were detected in feces from all cows fed transgenic feed.	Milk of dairy cows fed GE corn for 25 mo should be classified not different from milk of cows fed non-GE corn.	Guertler et al. (2010)
B. Pig study				
Large white × landrace cross-bred male 40-d-old pigs (n = 40) were fed 1 of the following treatments: 1) isogenic corn-based diet for 110 d (isogenic), 2) Bt corn-based diet (MON810) for 110 d (Bt), 3) isogenic corn-based diet for 30 d followed by Bt corn-based diet for 80 d (isogenic/Bt), and 4) Bt corn-based diet (MON810) for 30 d followed by isogenic corn-based diet for 80 d (Bt/isogenic).	Feed intake, growth, characteristics, and body composition. Heart, kidneys, spleen and liver weight and histological analysis. Blood and urine analysis.	No difference in overall growth, body composition, organ weight, histology and serum and urine biochemistry. A significant treatment × time interaction was observed for serum urea, creatinine, and aspartate aminotransferase.	Serum biochemical parameters did not indicate organ dysfunction; changes were not accompanied by histological lesions. Long-term feeding of GE maize did not adversely affect growth or the selected health indicators investigated.	Buzoianu et al. (2012a)
	Effect on intestinal microbiota	Counts of the culturable bacteria enumerated in the feces, ileum, or cecum were not affected by GE feed. Neither did it influence the composition of the cecal microbiota, with the exception of a minor increase in the genus *Holdemania*.	Feeding Bt corn to pigs in the context of its influence on the porcine intestinal microbiota is safe.	Buzoianu et al. (2012d)
	Hematological analysis, measurement of cytokine and Cry1Ab-specific antibody production, immune cell phenotyping, and *cry1Ab* gene and truncated Bt toxin detection	On d 100, lymphocyte counts were higher (P < 0.05) in pigs fed Bt/isogenic than pigs fed Bt or isogenic. Erythrocyte counts on d 100 were lower in pigs fed Bt or isogenic/Bt than pigs fed Bt/isogenic (P < 0.05). Neither the truncated Bt toxin nor the *cry1Ab* gene was detected in the organs or blood of pigs fed Bt corn.	Perturbations in peripheral immune response were thought not to be age specific and were not indicative of Th 2 type allergenic or Th 1 type inflammatory responses. No evidence of *cry1Ab* gene or Bt toxin translocation to organs or blood following long-term feeding.	Walsh et al. (2012b)
Large White × Landrace cross-bred male pigs (9 per treatment group) fed diet containing 38.9% GE or non-GE isogenic parent line corn for 31 d.	Growth performance, intestinal histology, and organ weight and function.	Short-term feeding of Bt MON810 corn to weaned pigs resulted in increased feed consumption, less efficient conversion of feed to gain, and a decrease in goblet cells/mum of duodenal villus. There was a tendency for an increase in kidney weight, but this was not associated with changes in histopathology or blood biochemistry.	The biological significance of these findings is currently being clarified in long-term exposure studies in pigs.	Walsh et al. (2012a)
	Effects on the porcine intestinal microbiota were assessed through culture-dependent and -independent approaches.	Fecal, cecal, and ileal counts of total anaerobes, Enterobacteriaceae, and Lactobacillus were not significantly different between pigs fed the isogenic or Bt corn-based diets. Furthermore, high-throughput 16S rRNA gene sequencing revealed few differences in the compositions of the cecal microbiotas.	*Bacillus thuringiensis* corn is well tolerated by the porcine intestinal microbiota.	Buzoianu et al. (2012c)
	Immune responses and growth in weanling pigs. Determined the fate of the transgenic DNA and protein in vivo.	Interleukin-12 and interferon gamma production from mitogenic stimulated peripheral blood mononuclear cells decreased in GE-fed pigs. Cry1Ab-specific IgG and IgA were not detected in the plasma of GE corn-fed pigs. The detection of the *cry1Ab* gene and protein was limited to the gastrointestinal digesta and was not found in the kidneys, liver, spleen, muscle, heart, or blood.	No evidence of *cry1Ab* gene or protein translocation to the organs and blood of weaning pigs. The growth of pigs was not affected by feeding GE corn. Alterations in immune responses were detected; however, their biologic relevance is questionable.	Walsh et al. (2011)

continued

Table 1. (cont.)

Large White × Landrace cross-bred female pigs (12) – Fed for approximately 143 d throughout gestation and lactation F_0 + 1 generation (offspring at birth). Large White × Landrace cross-bred pigs (10) – Corn dietary inclusion rate identical between treatments (isogenic parent line corn from service to weaning and GE corn from service to weaning [Bt]) and ranged from 86.6% during gestation to 74.4% during lactation). Offspring (72) fed in 4 dietary treatments as follows: 1) non-GE corn-fed sow/ non-GE corn-fed offspring (non-GE/non-GE), 2) non-GE corn-fed sow/GE corn-fed offspring (non-GE/GE), 3) GE corn-fed sow/non-GE corn-fed offspring (GE/non-GE), and 4) GE corn-fed sow/GE corn-fed offspring (GE/GE) for 115 d.	Hematological and immune functions to detect possible inflammatory and allergenic responses at various times. Attempts to detect Cry1Ab protein in blood and feces at various times.	Cytokine production similar between treatments. Some differences in monocyte, granulocyte, or lymphocyte subpopulations counts at some times, but no significant patterns of changes.	No indication for inflammation or allergy due to GE corn feeding. Transgenic material or Cry1Ab-specific antibodies were not detected in sows or offspring.	Buzoianu et al. (2012b)
	Pig growth performance, BW, and feed disappearance recorded at the time of each dietary change (at weaning [d 0] and on d 30, 70, and 100) and at harvest (d 115). At harvest, organ weight, histological observations, and cold carcass weight. Serum biochemistry.	No pathology observed in the organs. Offspring of sows fed Bt corn had improved growth throughout their productive life compared to offspring of sows fed non-GE corn, regardless of the corn line fed between weaning and harvest. Some minor differences in average daily gain, carcass and spleen weights, dressing percentage, and duodenal crypt depths for offspring from GE fed or in average daily feed intake for offspring from sows fed GE and for GE-fed pigs or in liver weight for pigs in the GE/GE.	Feeding GE Bt corn from 12 d after weaning to slaughter had no adverse effect on pig growth performance, body composition, organ weights, carcass characteristics, or intestinal morphology. Transgenerational consumption of GE corn diets not detrimental to pig growth and health.	Buzoianu et al. (2013a)
	Sequence based analysis of the intestinal microbiota of sows and their offspring fed GE corn	At d 115 postweaning, GE/non-GE offspring had lower ileal Enterobacteriaceae counts than non-GE/non-GE or GE/GE offspring and lower ileal total anaerobes than pigs on the other treatments. Genetically engineered corn-fed offspring also had higher ileal total anaerobe counts than non-GE corn-fed offspring, and cecal total anaerobes were lower in non-GE/GE and GE/non-GE offspring than in those from the non-GE/non-GE treatment. The only differences observed for major bacterial phyla using 16S rRNA gene sequencing were that fecal Proteobacteria were less abundant in GE corn-fed sows before farrowing and in offspring at weaning, with fecal Firmicutes more abundant in offspring.	While other differences occurred, they were not observed consistently in offspring, were mostly encountered for low-abundance, low-frequency bacterial taxa, and were not associated with pathology. Therefore, their biological relevance is questionable. This confirms the lack of adverse effects of GE corn on the intestinal microbiota of pigs, even following transgenerational consumption.	Buzoianu et al. (2013b)
	The effects of feeding GE corn during first gestation and lactation on maternal and offspring health serum total protein, creatinine and gamma-glutamyltransferase activity, serum urea, platelet count, and mean cell Hb concentration	Genetically engineered corn-fed sows were heavier on d 56 of gestation. Offspring from sows fed GE corn tended to be lighter at weaning. Sows fed GE corn tended to have decreased serum total protein and increased serum creatinine and gamma-glutamyltransferase activity on d 28 of lactation. Serum urea tended to be decreased on d 110 of gestation in GE corn-fed sows and in offspring at birth. Both platelet count and mean cell Hb concentration (MCHC) were decreased on d 110 of gestation in GE corn-fed sows; however, MCHC tended to be increased in offspring at birth.	There was a minimal effect of feeding GE corn to sows during gestation and lactation on maternal and offspring serum biochemistry and hematology at birth or BW at weaning.	Walsh et al. (2013)

[1]GE = genetically engineered; Bt = *Bacillus thuringiensis*; Hb = hemoglobin.

multigenerational studies should be reserved for cases where some reasonable doubt remains following a 90-d feeding trial triggered by a potential hazard identified in the compositional analysis of the GE crop or other available nutritional or toxicological data.

Field Datasets of Livestock Populations Fed with Genetically Engineered Feed

Although a small number of controlled long-term and multigenerational feeding trials of commercialized GE crops in food-producing species are available in the peer-reviewed literature, large numbers of livestock in many countries have been consuming GE feed for over 15 yr. Hence, a very large and powerful set of GE-fed target animal data has been quietly amassing in public databases. United States agriculture feeds billions of food-producing animals each year, with annual broiler numbers alone exceeding the current size of the global human population (Table 2). During 2011, less than 5% of U.S. animals within each of the major livestock sectors were raised for certified National Organic Program (**NOP**) markets that specifically prohibit the feeding of GE feed (Table 2). Given the increase in GE adoption rates between 2000 and 2013, it can be predicted that the vast majority of conventionally raised livestock in

Table 2. Organic livestock production statistics in the United States (2011)

Industry	Number of organic farms in the United States[1]	Number of animals on organic farms[1]	Total number of livestock animals in the United States[2]	Organic livestock numbers as percent of the U.S. total[3]
Broilers	153	28,644,354	8,607,600,000	0.33%
Layers	413	6,663,278	338,428,000	1.97%
Turkeys	70	504,315	248,500,000	0.20%
Beef cows	488	106,181	30,850,000	0.34%
Dairy cows	1,848	254,711	9,150,000	2.78%
Hogs	97	12,373	110,860,000	0.01%

[1]USDA National Agricultural Statistics Service, 2012.

[2]USDA Economics, Statistics, and Market Information System, 2013.

[3]USDA Economic Research Service, 2013.

the United States consumed feed derived from GE crops over the past decade. Cumulatively, this amounts to over 100 billion animals consuming some level of GE feed between 2000 and 2011 (Table 3).

The duration and level of exposure to GE feed would be expected to vary depending on the animal industry. For example, in a typical U.S. broiler operation, chickens are fed for 42–49 d on diets that are composed of approximately 35% soybean meal and 65% corn grain, whereas in others species, longer-term exposure would be the norm (e.g., dairy cows over recurrent lactations). The average U.S. dairy cow has a productive life of 5 yr with 3 conceptions, 3 gestations, and 3 lactations. A typical U.S. dairy diet contains 50% corn silage, 20% corn grain, and 10% dehulled soybean meal. Also, many cows receive large portions of their rations as ground corn grain, fuzzy cottonseed (no processing except for removal of the lint), or roasted full-fat soybeans. Other GE sources of animal feed include alfalfa hay, sugar beet pulp, corn distillers grains or other coproducts from corn processing, cottonseed meal, canola meal, and soy hulls. A beef cow on the range might consume only some GE alfalfa hay, but her progeny entering the feedlot might be expected to consume a ration containing high quantities of GE feed during their 120 d in the feedlot before harvest. Depending on the feeding stage and relative feed prices, feedlot rations will consist of about 80 to 85% grain (usually corn); distillers' grains and/or other sources of starch/energy; and 10 to 15% hay, silage, or other forage. The remaining share of the ration will include some protein source such as soybean or cottonseed meal (Mathews and Johnson, 2013), also likely to be of GE origin.

Table 3. Estimated cumulative number of livestock raised in the United States during the period from 2000 to 2011

Industry[1]	United States
Broilers	94,683,600,000
Layer Hens	3,722,708,000
Turkeys	2,733,500,000
Beef cattle	339,350,000
Dairy Cows	33,550,000
Hogs	1,219,460,000
Total	102,732,168,000

[1]Numbers for broilers, hogs (barrows and gilts), and beef cattle (steers) are for slaughtered animals during calendar year. Dairy animals are number of dairy cows in a calendar year divided by 3 to account for 3 lactations per animal.

It would be reasonable to hypothesize that if animal feed derived from GE crops had deleterious effects on animals consuming GE feed, then animal performance and health attributes in these large commercial livestock populations would have been negatively impacted. To examine this hypothesis further, in October 2013, data on livestock health were collated from publicly available sources in the United States from before the introduction of GE crops in 1996 through 2000 through 2011, a decade when high levels of GE ingredients would be expected to be present in livestock feed based on the known extent of GE crop cultivation. Data were collected for the broiler, dairy, hog, and beef industries. In general, USDA data sets were from the Economics, Statistics, and Market Information System (2013). Additional data for broilers were available from the National Chicken Council (2011) and were 1) days to market, 2) feed efficiency (feed to meat gain ratio), and 3) percent mortality.

Yearly data on cattle condemnation rates were available for 1999 through 2002 from the USDA Food Safety and Inspection Service (**FSIS**) website (USDA Food Safety and Inspection Service, 2003) and from 2003 through 2007 based on a Freedom of Information Act request as reported (White and Moore, 2009). Data from 1994 was collected from the National Non-Fed Beef Quality Audit as reported (Boleman et al., 1998). Non-fed beef is from culled cows and bulls (i.e., animals that do not spend a significant amount of time being "fed" in a feedlot). Data were analyzed to compare trends before and after the introduction of GE feed into livestock diets. Regression analyses were performed for the period 1983 through 1994 as representative of a period with no GE feed and for the period from 2000 through 2011 as a period with high levels of GE feed based on high rates of GE crop adoption. Where data were available for both time periods, the slope of the regression lines between periods was compared using an unpaired *t* test.

Table 4. Livestock production statistics in the United States before and after the introduction of genetically engineered feed in 1996

Year	Milk yield, kg	Somatic cell count, cells/mL, 1,000s	Carcass wt, kg, broiler	Carcass wt, kg, hog	Carcass wt, kg, cattle	Broiler				Cattle postmortem condemned, %			
										Fed cattle		Non-fed cattle	
						Condemned, %	Market age, d	Feed to gain	Mortality rate, %	Steers	Heifers	Cows	Bulls
1983	5,708		1.82	75.3	318.8	1.54							
1984	5,667		1.85	75.7	317.5	1.60							
1985	5,910		1.87	76.6	329.3	1.74	49	5	2				
1986	6,029		1.89	77.1	327.4	1.90							
1987	6,252		1.91	77.6	325.2	1.91							
1988	6,446		1.92	78.5	330.2	1.95							
1989	6,460		1.93	78.0	336.1	1.95							
1990	6,640		1.95	79.4	336.1	1.83	48	5	2				
1991	6,742		1.97	79.8	343.3	1.87							
1992	6,995		2.01	79.8	344.7	1.72							
1993	7,054		2.03	81.2	338.8	1.58							
1994	7,315		2.06	81.6	351.9	1.68						2.6	
1995	7,461	304	2.08	82.1	348.8	1.79	47	5	1.95				
1996	7,485	308	2.12	82.1	347.4	1.80							
1997	7,671	314	2.14	83.9	346.5	1.82							
1998	7,797	318	2.16	83.9	357.8	1.86				0.09	0.10	2.22	0.26
1999	8,059	311	2.22	84.8	359.6	1.74				0.11	0.20	2.11	0.31
2000	8,256	316	2.22	86.6	361.9	1.56	47	5	1.95	0.13	0.17	2.71	0.32
2001	8,226	322	2.24	87.5	361.9	1.31				0.09	0.10	2.67	0.31
2002	8,422	320	2.28	87.5	373.2	1.07				0.08	0.09	2.77	0.24
2003	8,503	319	2.31	88.0	359.2	1.00				0.09	0.08	2.92	0.75
2004	8,597	295	2.34	88.0	361.0	1.13				0.08	0.08	2.44	0.35
2005	8,878	296	2.39	89.3	370.5	1.04	48	4	1.95	0.07	0.07	2.59	0.30
2006	9,048	288	2.44	89.8	377.8	1.22	48	5	1.96	0.06	0.07	2.34	0.30
2007	9,191	276	2.45	89.8	376.4	1.16	48	4.5	1.95	0.05	0.06	2.21	0.28
2008	9,250	262	2.48	89.8	380.0	1.10	48	4.5	1.93				
2009	9,332	233	2.48	90.7	384.1	0.91	47	4.1	1.92				
2010	9,591	228	2.53	91.2	378.7	0.88	47	4.0	1.92				
2011	9,680	217	2.58	92.1	381.4	0.87	47	3.8	1.91				

Livestock production statistics for the United States before and after the introduction of GE feed crops in 1986 are summarized in Table 4. In all industries, there were no obvious perturbations in production parameters over time. The available health parameters, somatic cell count (an indicator of mastitis and inflammation in the udder) in the dairy data set (Fig. 1), postmortem condemnation rates in cattle (Fig. 1), and postmortem condemnation rates and mortality in the poultry industry (Fig. 2) all decreased (i.e., improved) over time.

All animals arriving at USDA-inspected slaughter facilities undergo both antemortem and postmortem inspections to identify abnormalities. Carcasses are condemned postmortem if there are visible lesions or tumors present on organs and carcasses. Of the more than 163 million cattle arriving at USDA-inspected slaughter facilities for the years 2003 through 2007, a total of 769,339 (0.47%) were condemned (White and Moore, 2009). Cattle fed or finished in feedyards, typically for 120 d before slaughter on high concentrate diets containing corn and soy as major ingredients, made up the majority (82%) of the cattle at harvest but represented a minority (12%) of the cattle condemned. Condemnation rates for non-fed cattle, particularly cows, were higher than for fed cattle, but the rate in 2007 (2.49%), the last year for which data are available, was similar to that reported in cattle in 1994 (2.6%; Boleman et al., 1998), before the introduction of GE crops.

The broiler data are particularly important due to the large number of animals involved (approximately 9 billion broilers are processed annually in the United States) and the fact that there are several variables that are indicative of health (Fig. 2). The rate of broiler carcass condemnation decreased significantly over time and was at its lowest in 2011. Moreover, mortality was essentially unchanged throughout the years presented and was also at its lowest in 2011. Although broilers are exposed to large amounts of corn and soybean meal during their 42- to 49-d lifespan, they increase their body size 60-fold during this period, making them very sensitive to

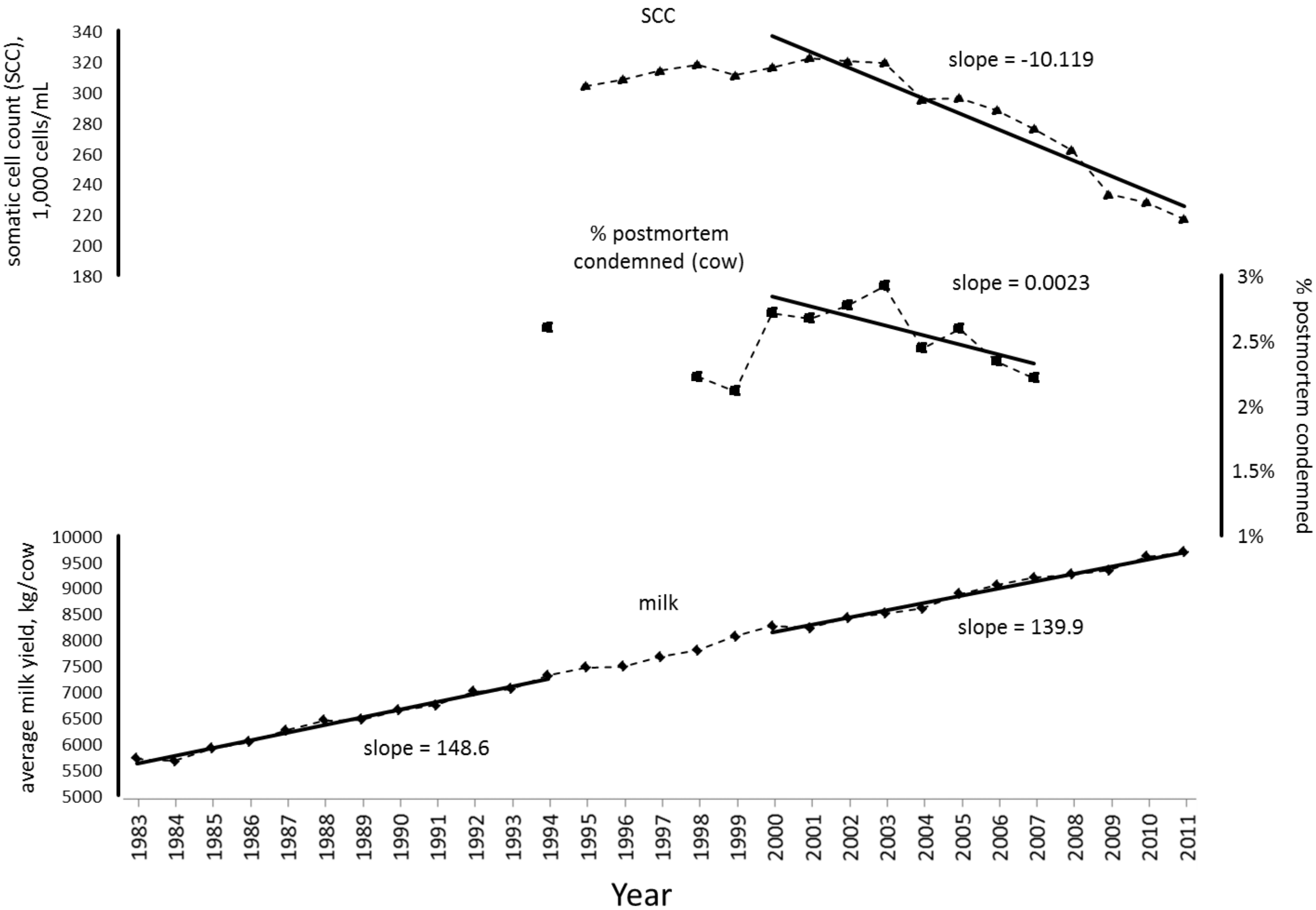

Figure 1. Milk production, percent postmortem condemned, and somatic cell counts for the United States before and after the introduction of genetically engineered crops in 1996. Sources: USDA National Agricultural Statistics Service, 2013; USDA Food Safety and Inspection Service, 2003; White and Moore, 2009; Boleman et al. (1998). Slope does not differ significantly between time periods 1983 through 1994 and 2000 through 2011.

dietary perturbations (European Food Safety Authority, 2008; International Life Sciences Institute, 2003). The conversion of feed to gain continuously decreased from 5 in 1985 to 3.8 in 2011, attributable most likely to improved genetics (Havenstein et al., 2003) and management, but this ratio is something that would be expected to worsen (i.e., increase) if the health of these animals was deteriorating following exposure to GE feed. An estimated 24 consecutive generations of broilers would have been consuming GE feed during the time period 2000 to 2011.

These field data sets representing billions of observations did not reveal unfavorable or unexpected trends in livestock health and productivity. The available health indicators from U.S. livestock suggest that these rates actually improved over time despite widespread adoption of GE crops in U.S. agriculture and increasing levels of GE content in livestock diets. There was no indication of worsening animal health after the introduction of GE feed, and productivity improvements continued in the same direction and at similar rates as those that were observed before the introduction of GE crop varieties in 1996.

A small number of experimental animal feeding studies have generated highly controversial results suggesting deleterious health effects of GE feed. Some of these reports were published and then retracted (Séralini et al., 2012), although recently and controversially republished without further peer review (Séralini et al., 2014), and others were never subjected to peer review (Ermakova, 2005; Velmirov et al., 2008). Adverse effects, including high rates of tumorogenesis, sterility, premature mortality, and histopathological abnormalities have been reported. These studies have been criticized for nonadherence to Organisation for Economic Co-operation and Development (Paris, France) consensus documents and standard protocols. Methodological flaws variously include the use of control feed that was not derived from near-isogenic lines, insufficient animal numbers to enable appropriate statistical power, lack of dose response or insufficient or no information on natural variations in test parameters, overinterpretation of differences that lie within the normal range of variation (i.e., the biological significance of differences is more important than their mere presence), and poor toxicological and/or statistical

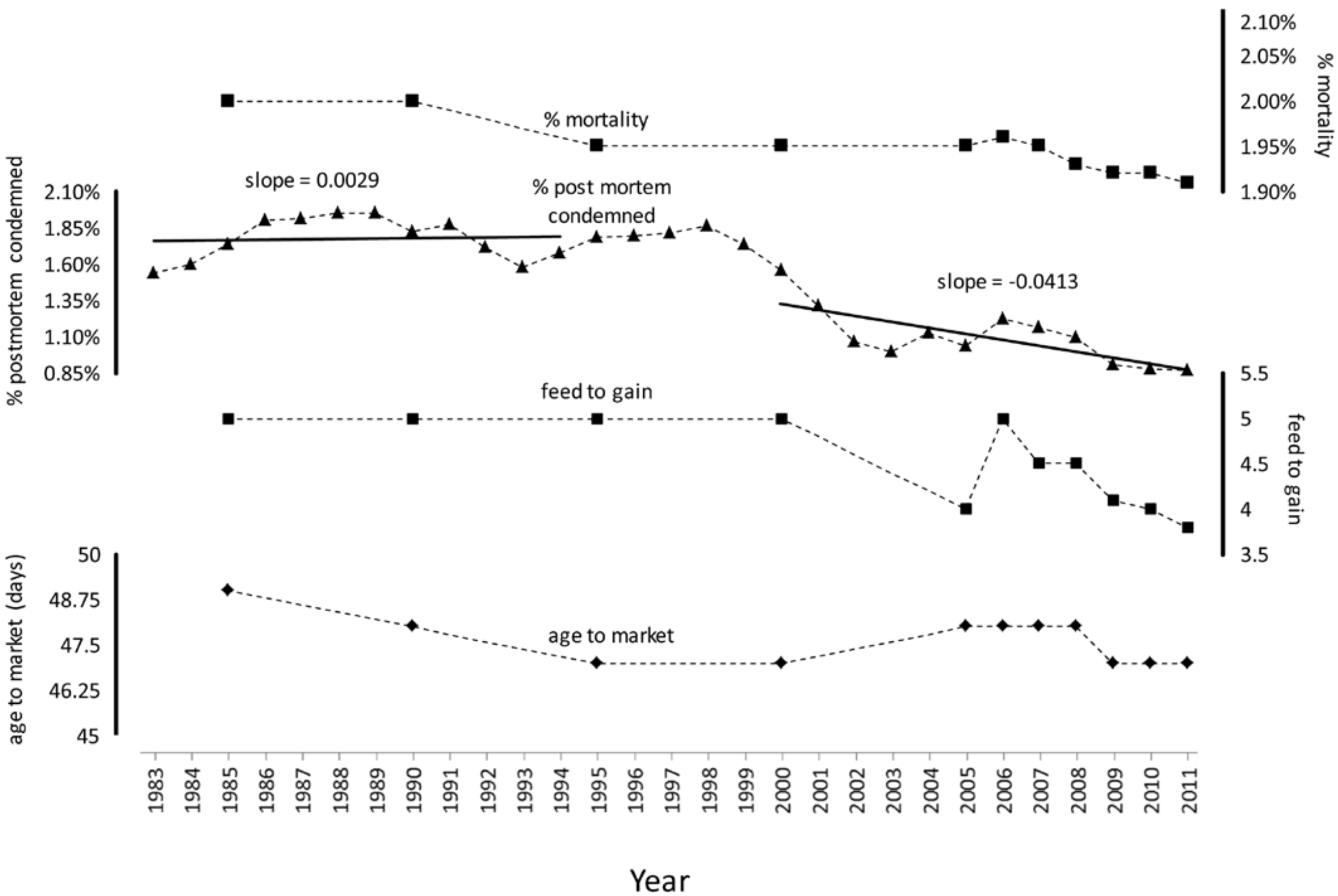

Figure 2. United States broiler statistics before and after the introduction of genetically engineered crops in 1996. Sources: USDA National Agricultural Statistics Service, 2013; National Chicken Council, 2011. Slope differs between time periods 1983 through 1994 and 2000 through 2011 (*$P < 0.05$).

interpretation of the data (Bartholomaeus et al., 2013; European Food Safety Authority, 2012; Marshall, 2007; Schorsch, 2013; The Australian and New Zealand Food Standards Agency, 2013, 2012). A particularly succinct summary of the methodological design flaws is presented in Table 5 (Bartholomaeus et al., 2013).

Despite a wealth of studies and literature to the contrary, these isolated and poorly designed studies have resulted in the promulgation of new regulations, including a mandatory 90-d rodent subchronic toxicity feeding study for all new GE approvals in the EU (Kuiper et al., 2013), and have generated a great deal of media attention (Arjó et al., 2013). They are also contrary to the field experience as documented by the health and production data collected on the billions of commercial food-producing animals that have primarily been consuming GE feed for over a decade. The media attention devoted to these sensational studies is exacerbating the continued controversy associated with the safety of GE food and feed and is bolstering arguments calling for the mandatory labeling of milk, meat, and eggs from GE-fed animals.

Summary of Data on Recombinant DNA/protein in Milk, Meat, and Eggs from Animals Fed Genetically Engineered Feed

Studies have concluded that animals do not digest transgenic and native plant DNA differently and that rDNA from GE crops has not been detected in animal products (Einspanier, 2013). Fragments of highly abundant plant DNA (e.g., chloroplast genomes) have been found in the digestive tracts and tissues of some species (Einspanier et al., 2001); however, neither recombinant DNA nor protein has ever been found in milk, meat, or eggs from animals that have eaten GE feed with the exception of a single study that reported the presence of fragments of transgenic DNA in both "organic" and "conventional" milk in Italy (Agodi et al., 2006). The organic milk was derived from animals not fed GE crops, so the authors postulated that the rDNA was due to feed and fecal contamination during milking of cows offered GE diets. This result has not been repeated despite recent studies using more sophisticated techniques that have looked for the presence of transgenic material in animal products (Buzoianu et al., 2012b; Deb et al., 2013; Guertler et al., 2010; Tufarelli and Laudadio, 2013). It is important to note that animals and humans regularly ingest DNA and

Table 5. Examples of limitations in experimental design, analyses, and interpretation in some whole food toxicity studies with genetically engineered (GE) crops (Bartholomaeus et al., 2013). Table reproduced with permission

Best practices	Deficiencies observed	References
Experimental design		
Identity of test and control substances	The identity of the GE test substance was not confirmed through an appropriate analytical method. Confirmation of correct control and test crop presence in diet was not conducted.	Brake and Evenson (2004), Ermakova (2005), Ewen and Pusztai (1999), Kilic and Akay (2008), and Malatesta et al. (2002a,b, 2003, 2005, 2008)
Use of appropriate control crops	The control crop was not of similar genetic background to the GE test crop. In some studies the control was simply identified as a “wild” variety.	Ermakova (2005), Ewen and Pusztai (1999), Malatesta et al. (2002a,b, 2003, 2005, 2008), and Rhee et al. (2005)
	The test and control substances were not produced under similar environmental conditions and/or no information was provided on the production of test and control substances.	Ermakova (2005), Ewen and Pusztai (1999), and Malatesta et al. (2002a,b, 2003, 2005, 2008)
Acceptable levels of contaminants (e.g., pesticides, mycotoxins, other microbial toxins) in control and test crops	Study results were not interpreted in light of differences in antinutrient or mycotoxin levels in test and control diets.	Carman et al. (2013) and Velmirov et al. (2008)
Nutritionally balanced diet formulations for control and test diets	Compositional analyses were not performed on the test and control substances to confirm that test and control diets had similar nutrient content and were nutritionally balanced.	Ewen and Pusztai (1999)
Description of study design, methods, and other details sufficient to facilitate comprehension and interpretation	Inadequate information was provided on the source of animals used, age, sex, animal husbandry practices followed, collection, and evaluation of biological samples to confirm that the procedures followed met accepted practices.	Ermakova (2005), Ewen and Pusztai (1999), and Séralini et al. (2012, 2014)
Statistical analyses and study interpretation		
Use of appropriate statistical methods for the design of the study	Statistical methods were sometimes not provided in sufficient detail to confirm if they were conducted appropriately for the data that were collected; statistical methods were documented but were not appropriate. Estimates of statistical power were based on inappropriate analyses and magnitudes of differences.	de Vendomois et al. (2009), Ewen and Pusztai (1999), Malatesta et al. (2003, 2005), and Séralini et al. (2007, 2012, 2014)
Appropriate interpretation of statistical analyses	Statistical differences were not considered in the context of the normal range for the test species, including data from historical and/or concurrent reference controls; the toxicological relevance of the difference was not considered (i.e., the reported finding is not known to be associated with adverse changes). Observed differences were not evaluated in the context of the entire data collected to determine if changes in a given parameter could be correlated with changes in related parameters.	Carman et al. (2013), de Vendomois et al. (2009), Ewen and Pusztai (1999), Kilic and Akay (2008), Malatesta et al. (2002a,b, 2003, 2005), and Séralini et al. (2007, 2012, 2014)
Adequate numbers of animals or test samples collected to be able to make meaningful comparisons between test and control groups	Too few animals/group were used to make meaningful comparisons; tissue sampling did not follow acceptable guidelines and was too limited to provide an accurate assessment of what was occurring in the organ being examined.	Ermakova (2005), Malatesta et al. (2002a,b, 2003, 2008), and Séralini et al. (2012, 2014)
Study publication and availability		
Publication of studies in peer-reviewed journals	Circumvention of the peer-review process removes a level of review that may contribute to ensuring that WF studies are appropriately designed and interpreted.	Ermakova (2005) and Velmirov et al. (2008)

RNA as part of traditional diets without consequence. The DNA from GE crops is chemically equivalent to DNA from other sources and both are thoroughly broken down in the gastrointestinal tract during digestion (Beever and Kemp, 2000; Jonas et al., 2001; CAST, 2006).

Intact recombinant proteins have never been detected in tissues or products of animals fed GE crops (Alexander et al., 2007). This is particularly important when considering the prospect of labeling secondary products such as milk, meat, and eggs. In some countries, mandatory food labeling regulations target the presence of GE components in the finished product (e.g., Australia, New Zealand, and Japan), whereas in other countries, regulations target foods that use GE technology as a part of the production process (e.g., the EU, Brazil, and China). It should be noted, however, that only Brazil currently requires mandatory labeling of products from animals that consume GE feed. Technically, the Brazilian law requires the label to state “(name of animal) fed with rations containing a transgenic ingredient” or “(name of ingredient) produced from an animal fed with a ration containing a transgenic ingredient.”, but has yet to fully implement these laws. Given that there are no detectable and reliably quantifiable traces of GE materials in milk, meat, and eggs, any proposed labeling of animal products derived from GE-fed livestock would have to be based on documenting the absence of GE crops in the production chain, thereby necessitating the need for identity preservation and segregation requirements for producers and importers (Bertheau et al., 2009). This difference is important for verification: a product-based system can be enforced with testing equipment to analyze for the presence of GE materials and can filter a cheater, whereas a tracking system segregating indistinguishable products cannot guarantee the absence of products from animals that might have eaten GE feed (Gruère and Rao, 2007).

In 2012 the USDA's FSIS approved a voluntary process-based label for meat and liquid egg products that allows companies to label that they meet the Non-GMO Project's standard (<0.9% tolerance for GE presence) for the avoidance of GE feed in the diet of the animal producing the product. The FSIS allows companies to demonstrate on their labels that they meet a third-party certifying organization's standards, provided that the claims are truthful, accurate, and not misleading. A similar approach of certifying the absence of prohibited methods in the production chain, rather than testing for some quantifiable attribute in the end product, is used for other voluntary process-based labels such as certified organic and the USDA's Agricultural Marketing Service (**AMS**) Process Verified Never Ever 3 (**NE3**) Program which requires that animals are never treated with antibiotics or growth promotants or fed animal byproducts. Again, because the products raised using these methods are indistinguishable from conventional animal products, the USDA Process Verified Program ensures that the NE3 requirements are supported by a documented quality management system.

2013 Data on Global Production and Trade in Genetically Engineered Feedstuffs and Sources of Non-Genetically Engineered Feedstuffs

Global grain production is currently 2.5 billion t, of which approximately 12% (300 million t) is traded. Soy and corn make up two-thirds of global grain trade and these are the main players in commercial animal feed. Figure 3 illustrates the major global producers of these 2 crops and the proportion of global production that is from GE crop varieties. It is estimated that approximately 85% of soybean and 57% of corn grain production (USDA Foreign Agricultural Service, 2014b) are used in global livestock diets annually. The demand for livestock products has been increasing in response to population growth and income, particularly in developing countries. In Asia alone, consumption of meat and dairy products has been increasing annually by approximately 3 and 5%, respectively (Food and Agriculture Organization of the United Nations, 2012). Increase in demand for animal products, especially meat, will drive demand for grain and protein feeds (USDA Economic Research Service, 2008). The Food and Agriculture Organization of the United Nations (Rome, Italy) predicts that by 2050 global grain trade will double to 600 million t (Bruinsma 2009).

Of the protein sources available, soybean meal has one of the best essential AA profiles for meeting the essential AA needs of livestock and poultry. It is a good source of both lysine and methionine, which are the first limiting AA for swine and poultry, respectively. It is estimated that 79% (85 million ha) of global soybean hectarage is planted to GE varieties (Fig. 3). In 2013, 36.5% of global soybean production (97.2 million t) was exported and 97% came from 3 countries that grow GE soybeans—the United States, Brazil, and Argentina (Fig. 4).

Soybean meal is also an important component of animal feed globally (Fig. 5). In the 2011 to 2012 marketing year, domestic animal agriculture used 27.6 million t of U.S. soybean meal. Poultry continue to be the single largest domestic user of soybean meal, consuming about half of all meal, followed by swine. Soybean meal is a very important protein source for animal feeds in the EU, supplying 46% of the lysine supply overall. The EU imports 65% of its protein-rich feedstuffs, for which there are no alternative sources grown in the EU (Popp et al., 2013), and is the largest importer of soybean meal and the second largest importer of soybeans after China (Fig. 4 and 5). About 70% of soybean meal consumed in the EU is imported and 80% of this meal is produced from GE soybeans.

Corn is an important subsistence crop in many parts of the world and hence the majority of production is consumed within the country of production. Although only 32% (57 million ha) of global corn hectarage is planted with GE varieties (Fig. 3), 71% of global trade came from those countries that grow GE corn varieties (Fig. 6). Approximately 11.6% (100 million t) of global corn production was internationally traded in 2013. Three of the top 5 corn exporting countries—the United States, Brazil, and Argentina—currently grow GE corn. The remaining 2 countries—Ukraine and India—do not have officially registered and approved GE corn varieties.

Of the top 5 corn importing countries—Japan, Mexico, the EU, South Korea, and Egypt—only 5 countries within the EU (Spain, Portugal, Romania, Czechoslovakia, and Slovakia) grew a small amount (148,013 ha) of Bt-MON810 corn (USDA Foreign Agricultural Service, 2014a). Corn is the second largest category of GE products imported into the EU after soy. Unlike soybean, EU corn production is sufficient to meet most of its own corn consumption, with imports accounting for only 10% of total supply. Annual EU imports of corn products include US$1.8 billion of corn, $151 million of corn seed for planting, and $87 million of dried distillers grains (USDA Foreign Agricultural Service, 2013a).

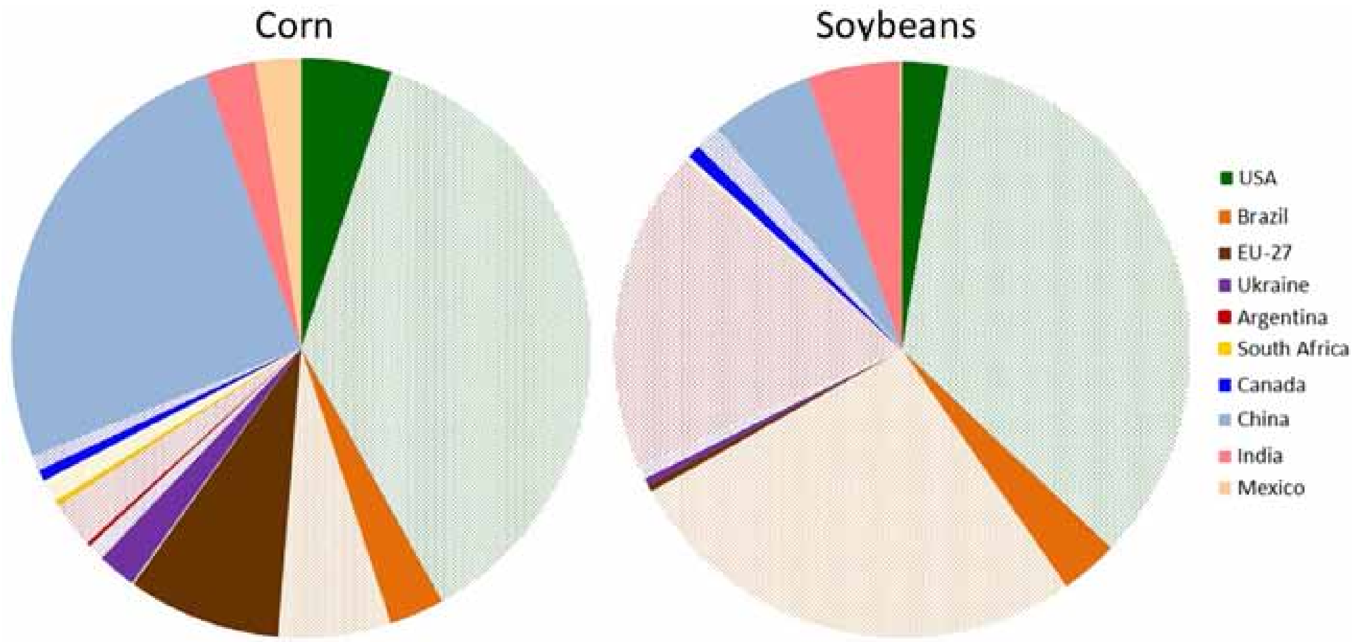

Figure 3. Genetically engineered (GE) and conventional corn and soy produced (million t) by selected countries 2012. Pattern represents production from GE varieties and solid slices represent conventional varieties. Sources: United States Department of Agriculture Foreign Agricultural Service; individual country Global Agricultural Information Network reports 2013; Food and Agriculture Organization of the United Nations (FAOSTAT). EU-27 = the 27 member states of the European Union (EU); production and trade database searches (faostat3.org/faostat-gateway/go/to/download/Q/*/E).

Prevalence of Markets Sourcing Non-Genetically Engineered Feed Globally for Livestock Populations as Compared to Conventional

World markets for grains can be separated into 4 segments: the conventional market (non-GE grain that is not certified as such), the mixed market (GE and conventional undifferentiated), the identity-preserved (certified non-GE) market, and the organic market. It is difficult to determine exact size estimates for these different markets, although it can be stated that the conventional and mixed markets are much larger than the remaining 2.

Of the top 5 soybean meal exporting countries in 2013—Argentina, Brazil, the United States, India, and Paraguay—only India does not allow the cultivation of GE soybeans. Of the top 5 soybean meal importing countries in 2013—the EU, Indonesia, Thailand, Vietnam,

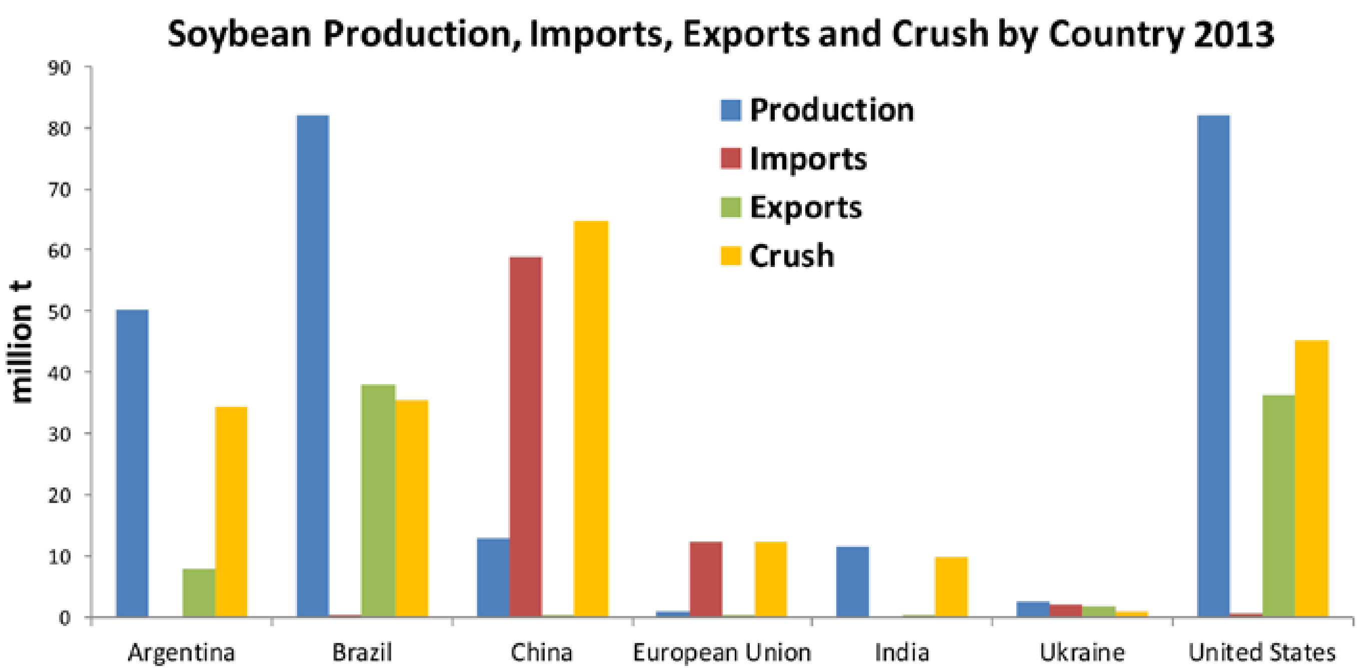

Figure 4. Soybean production, imports, exports, and crush (million t) by major import and export countries, 2013. Source: United States Department of Agriculture Foreign Agricultural Service; Production and trade database searches (http://faostat3.fao.org/faostat-gateway/go/to/download/G1/*/E).

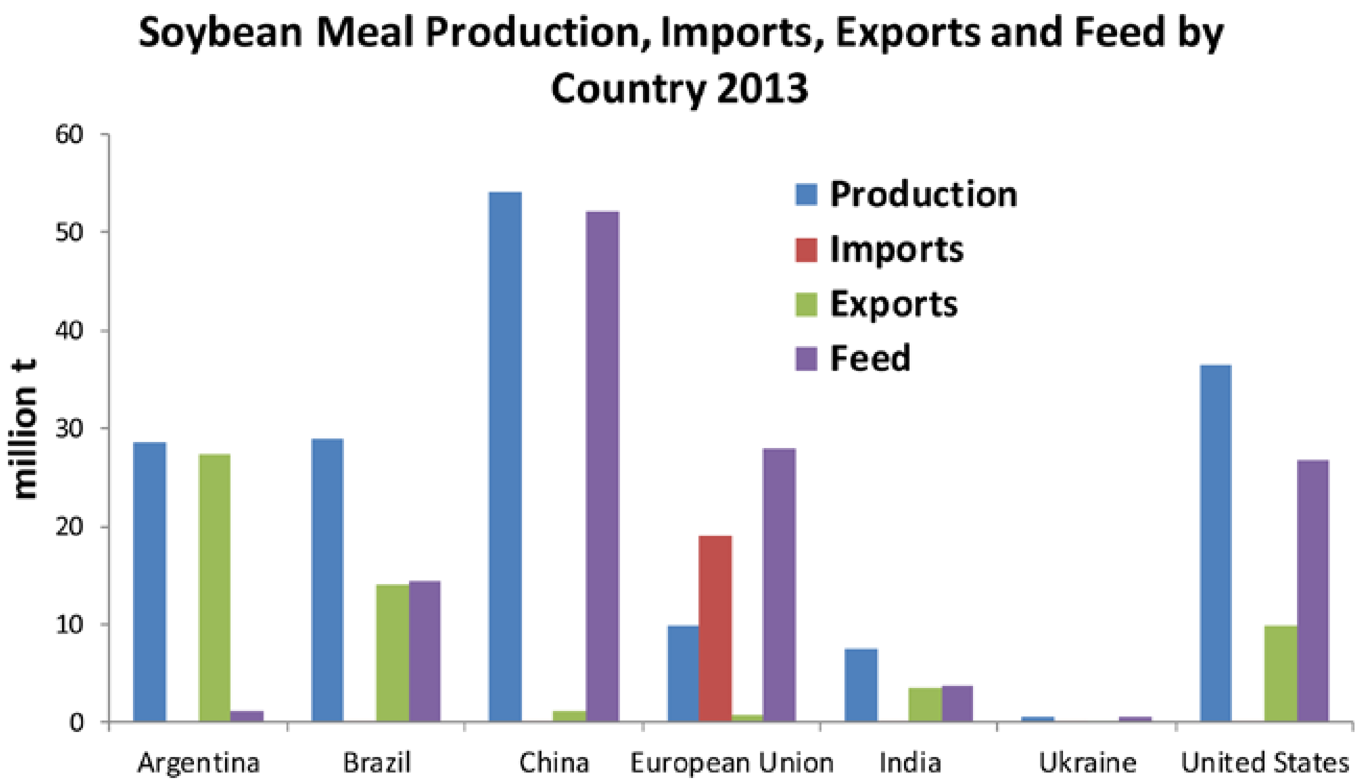

Figure 5. Soybean meal production, imports, exports, and feed (million t) by major import and export countries, 2013. Source: United States Department of Agriculture Foreign Agricultural Service; production and trade database searches (http://faostat3.fao.org/faostat-gateway/go/to/download/G1/*/E).

and Iran—none grow GE soybeans (USDA Foreign Agricultural Service, 2014a). It is estimated that between 4.0 and 4.5% of global trade in soybeans is required to be identity-preserved certified non-GE, and if it is assumed that this volume of traded soybeans is segregated from supplies that may contain GE soybeans, then the GE share of global trade is in the range of 93 to 96% (Table 6). A similar pattern occurs in soybean meal, where 88% of globally traded meal likely contains GE material (Table 7).

The estimated size of the export market requiring certified non-GE corn is 7.3 million t or 7% (Table 6). This excludes countries with markets for certified non-GE corn for which all requirements are satisfied by domestic production (e.g., corn in the EU). Farm animal feed in the 27 member states of the European Union (EU-27) is composed of 50% roughages and 10% grains produced on farm, 10% purchased feed materials, and 30% industrial compound feed. It has been estimated that in the EU, less than 15% of the animal feed market is identity-preserved certified non-GE, although there are great variations between countries. The main driver for non-GE feed is the poultry sector (17%) followed by the cattle (9%) and pig sectors (2%; European Feed Manufacturers' Federation, 2013).

The United States used to be a major supplier of corn to the EU in the 1990s but GE corn plantings in the United States caused a drastic decline in corn exports to the EU because of trade disruptions due to asynchronous approvals (i.e., cultivation approvals of specific GE varieties in the United States occurring before food and feed import approvals in the EU). The result is that the United States is no longer a major supplier of corn to the EU. Similarly, in 2007 there was a problem with asynchronous approval of a GE corn variety approved for cultivation in Argentina but unapproved for food and feed use in the EU. This concentrated demand on corn grown in Brazil, which increased prices an estimated €50/million t for compound feed producers in the EU (Popp et al., 2013).

China, which imported an estimated 5 million t of corn in 2013, making it the sixth largest corn importer, began rejecting shipments of U.S. corn in November 2013 after tests found a GE variety of corn that had been approved for cultivation in the United States, Argentina, and Brazil since 2011 but was not approved for food and feed import into China, despite a 2010 regulatory submission requesting such approval. China has a zero-tolerance policy for unapproved events. Since these trade disruptions began, a total of 3.3 million t of U.S. corn have been subject to rejection and diverted shipments (1.4 million t) or canceled or deferred sales. It has been estimated that up to $2.9 billion in economic losses were sustained by the U.S. corn, distillers' grains, and soy sectors in the aftermath of the zero-tolerance enforcement policy on U.S. export shipments to China (National Grain and Feed Association, 2014).

Interestingly, Ukraine signed a 3-yr agreement with China in 2013 for the delivery of 4 to 5 million t of corn

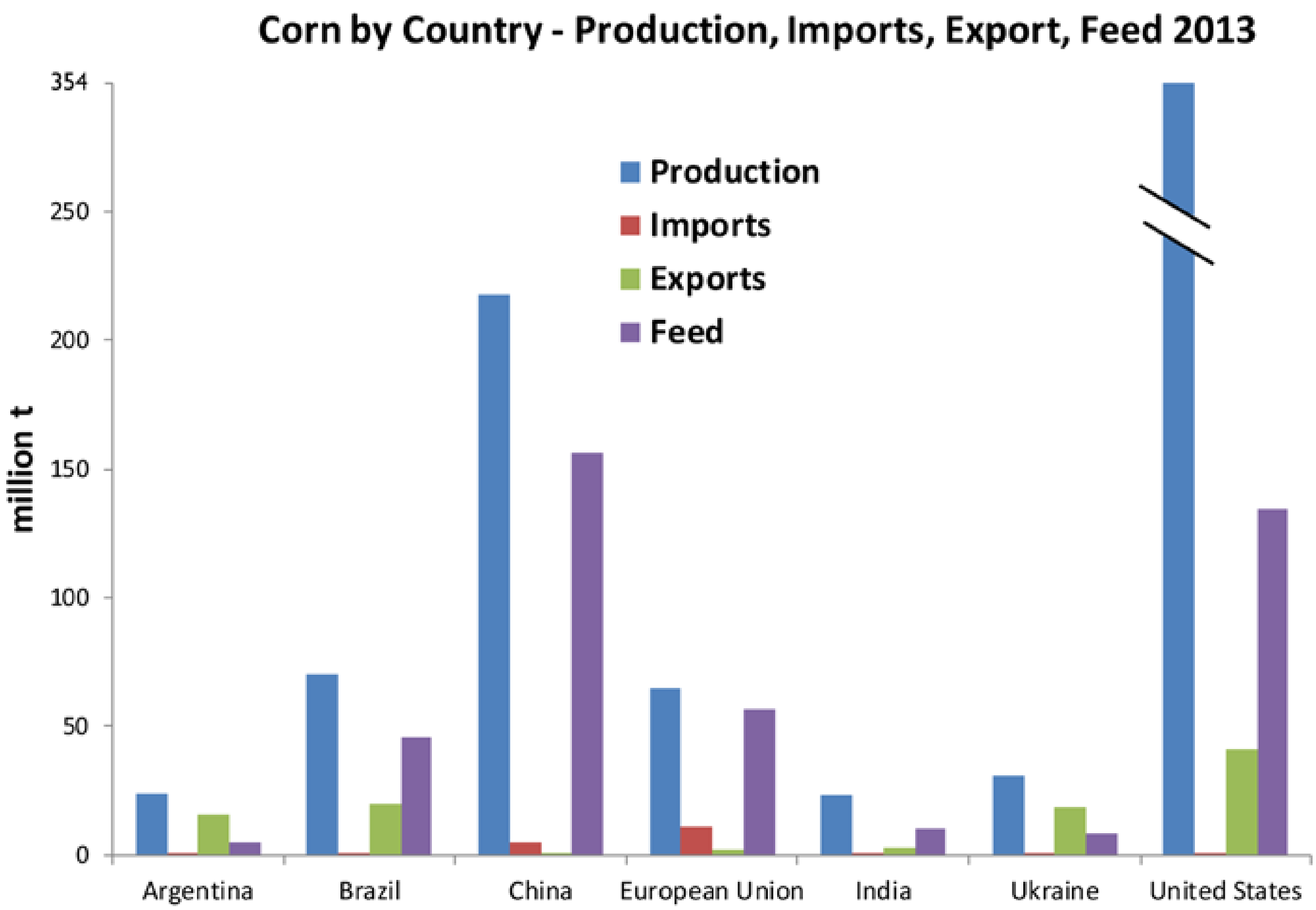

Figure 6. Corn production, imports, exports, and feed (production and trade database searches (http://faostat3.fao.org/faostat-gateway/go/to/download/G1/*/E)) by major import and export countries, 2013. Source: United States Department of Agriculture Foreign Agricultural Service; production and trade database searches(http://faostat3.fao.org/faostat-gateway/go/to/download/G1/*/E).

per year. Ukraine does not export or import GE products as none are officially registered and approved for commercial use or sale in the country. However, private sources estimate approximately 60% of the Ukraine soybean crop and 30% of the corn crop consist of GE varieties (USDA Foreign Agricultural Service, 2013b). China only accepts GE-positive cargo if the shipment is marked accordingly and contains only those GE events that are approved for import in China as well as cultivation in the country of origin. Given asynchronous regulatory approvals and the realities of agricultural production systems where harvesting machinery and storage facilities are shared among different production systems, trade disruption appears almost unavoidable if importing countries enforce a "zero-tolerance" policy for unapproved events that have been approved for cultivation in exporting countries.

Reliance on imported animal feed is becoming increasingly complicated for countries that wish to source non-GE products due to the significant GE adoption rate worldwide. In 2013, 4 major United Kingdom food supermarket groups—Tesco, Cooperative, Marks and Spencer, and Sainsbury's—ceased requiring that poultry and egg suppliers use only non-GE feed (Popp et al., 2013). Likewise, in 2014, the German poultry industry, which feeds 0.8 million t of soybean meal annually, abandoned its commitment to use only non-GE soybeans in poultry feed (USDA Foreign Agricultural Service, 2014c). This was largely due to the fact that Brazil is growing more GE soybeans and therefore has less identity-preserved certified non-GE soybeans available for export. As the global production of GE feed crops continues to rise, the EU's stringent GE tolerance levels (0.9% GE material limit plus 0.05% measuring uncertainty tolerance) and zero tolerance for unapproved events are complicating the maintenance of non-GE supply chains (Popp et al., 2013).

Current U.S. Options for Products from Non-Genetically Engineered Fed Livestock

Consumers wishing to purchase products from animals fed non-GE diets in the United States currently have that choice available through certified NOP products, the FSIS-approved Non-GMO Project verified label claim for meat and liquid eggs, and other non-genetically modified organism certification programs. Additionally, some private retailers are pursuing voluntary labeling.

Table 6. Share of global crop trade accounted for by genetically engineered (GE) crop production 2012/2013 (million t; Brookes and Barfoot, 2014c). Table reproduced with permission

Variable	Soybeans	Corn	Cotton	Canola
Global production	266	862.9	26.8	62.6
Global trade (exports)	97.2	100.1	10.0	12.0
Share of global trade from GE producers	94.6 (97.3%)	71.3 (71.2%)	6.9 (69%)	10.2 (85%)
Estimated size of market requiring identity-preserved (certified non-GE) market (in countries that have import requirements)[1]	4.0–4.5	7.3	Negligible	0.1
Estimated share of global trade that may contain GE (i.e., not required to be segregated)	90.1–93.2	64–92.8	6.9	10.1
Percentage of global trade that may be GE	92.75–95.9%	64–92.7%	69%	84.2–85%

[1]Estimated size of market requiring certified conventional in countries with import requirements excludes countries with markets for certified conventional for which all requirements are satisfied by domestic production (e.g., corn in the European Union [EU]). Estimated size of certified conventional market for soybeans (based primarily on demand for derivatives used mostly in the food industry): main markets: EU, 2.5 to 3.0 million t bean equivalents, and Japan and South Korea, 1 million t.

For example, in March 2013, the retail chain Whole Foods Market set a deadline that by 2018, animal products sold in its U.S. and Canadian stores must be labeled to indicate whether or not they came from animals that had consumed GE feed (Whole Foods Market, 2013). These voluntary process-based labels, in effect, verify that GE crops were not used in the production process, rather than testing for the presence of GE content in the animal products themselves as such products contain no detectable and quantifiable traces of GE materials.

Given the high rates of GE adoption in major feed crops, U.S. producers wishing to purchase non-GE feed for their livestock likely contract with growers or source identity-preserved (certified non-GE) or organic feed. In 2011, the United States had 1.26 million ha of certified organic cropland and 0.93 million ha of certified organic pasture and range (USDA National Agricultural Statistics Service, 2012). This translates into roughly 0.8 and 0.5% of total U.S. cropland and pasture/rangeland, respectively (Fig. 7). The availability and cost of certified organic feeds is a major challenge for U.S. organic livestock producers. The costs of certified organic feedstuffs are 2 to 3 times greater than non-organically-grown feeds (Hafla et al., 2013).

United States feed grain distributors and soy product manufacturers report sourcing organic soybeans from other countries. Organic farmers and handlers anywhere in the world are permitted to export organic products to the United States if they meet NOP standards and are certified by a USDA-accredited organic certification body. In 2007, USDA-accredited groups certified 27,000 producers and handlers worldwide to the U.S. organic standard, with approximately 16,000 in the United States and 11,000 in over 100 foreign countries (Grow and Greene, 2009). In 2007, approximately half of the accredited foreign organic farmers and handlers certified to NOP standards were in Canada, Italy, Turkey, China, and Mexico. Organic farming is often labor intensive, and developing countries with lower farm labor costs may have a competitive advantage in the production of some organic products.

In 2009, Canada was the main market for U.S. organic exports, while countries in Latin America, including Mexico, Brazil, Argentina, and Uruguay, along with China and other countries in Asia are major sources of organic imports (Grow and Greene, 2009). The countries with the fastest growth in organic production are those that produce organic products for export including China, Bolivia, Chile, Uruguay, and Ukraine. The amount of organic farmland increased well over 1,000% in these countries between 2002 and 2006, while organic farmland in Europe and North America showed slower (27–80%) expansion rates (Grow and

Table 7. Share of global crop derivative (meal) trade accounted by genetically engineered (GE) product 2012/2013 (million t; Brookes and Barfoot, 2014c). Table reproduced with permission

Variable	Soymeal	Cottonseed meal	Canola/rape meal
Global production	179.3	20.5	34.9
Global trade (exports)	57.2	0.6	5.6
Share of global trade from GE producers	50.4 (88%)	0.29 (46%)	3.6 (64%)
Estimated size of market requiring identity-preserved (certified non-GE) market (in countries that have import requirements)[1]	2.1	Negligible	Negligible
Estimated share of global trade that may contain GE (i.e., not required to be segregated)	48.3	0.63	3.6
Percentage of global trade that may be GE	84.4%	45%	64%

[1]Estimated size of certified conventional market for soymeal: European Union, 2 million t, and Japan and South Korea, 0.1 million t (derived largely from certified conventional beans referred to in Table 6).

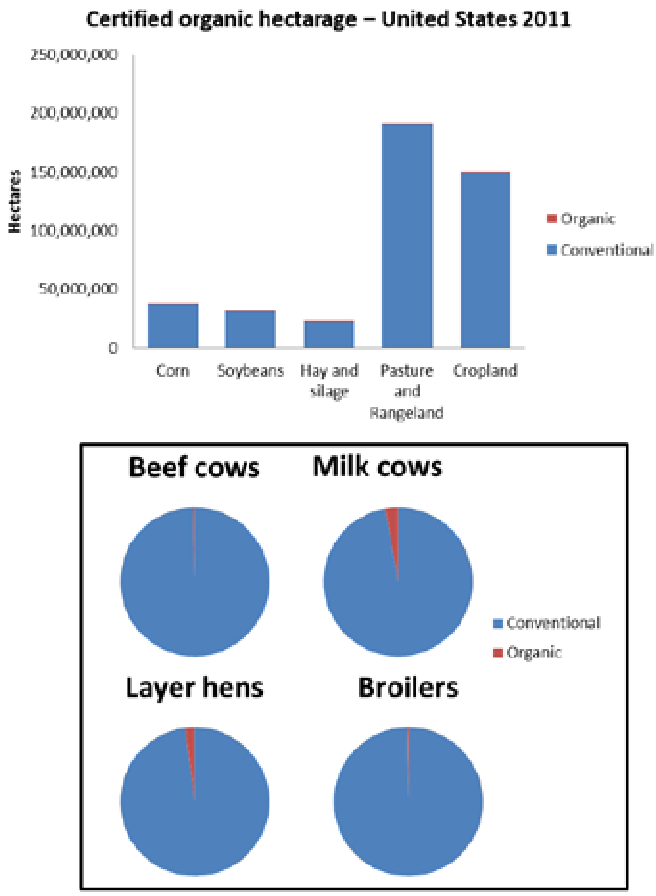

Figure 7. Certified National Organic Program hectarage and livestock numbers as a percentage of conventional U.S. numbers, 2011. Source: USDA National Agricultural Statistics Service, 2012. www.ers.usda.gov/datafiles/Organic_Produc tion/National_Tables_/CertifiedandtotalUSacreageselectedcropslivestock.xls. See online version for figure in color.

Greene, 2009). In 2013, the United States imported over $100 million of organic soybeans primarily from China and India (Fig. 8; Global Agricultural Trade System online [GATS] organic products www.fas.usda.gov/commodities/organic-products). The proportion of organic imports used for livestock feed versus human food purposes is unavailable as import product codes do not distinguish between these uses. Improved data collection is necessary to better describe international trade patterns in organic and identity-preserved (certified non-GE) feed.

Dairy

Organically raised livestock accounted for $1.31 billion in sales in 2011, the last year with a complete set of data on production and sales. Organic milk led livestock commodities, accounting for $765 million, or 58%, of organic animal product sales; however, less than 2% of U.S. dairy production is currently organic (Hafla et al., 2013). During 2011, approximately 254,700 dairy cows (2.78% of the total U.S. dairy herd; Table 2) on 1,848 dairy operations were certified organic. Production costs for organic dairies are greater than for conventional dairies due to the increased cost of organic feed and the increased use of labor and capital, which is not scale neutral as the total costs per unit of production drops sharply as herd size increases. Using pasture as a source of dairy forage is more common on organic dairies, which can help to reduce feed costs per cow but also contributes to lower production per cow. The U.S. organic dairy systems depend on the willingness of consumers to pay a premium (Hafla et al., 2013). The retail price for organic milk between 2004 and 2007 averaged 3 times the cost of conventional milk (USDA Economic Research Service, 2012b), and in 2013, organic milk made up 4.38% of total U.S. fluid milk market sales.

Beef

Natural, organic (grain-fed or otherwise), and grass/forage-fed (including cattle finished on grasses/forages to a specific quality standard) account for about 3% of the U.S. beef market (Mathews and Johnson, 2013). The term "natural" is not associated with an official production process standard so natural beef may come from animals that have consumed GE feed. Likewise, the USDA NE3 Process Verified Program does not mandate or specify the use of non-GE feed.

Beef from grass-fed ruminants can be labeled with a "grass (forage) fed" marketing claim through the AMS Process Verified Program if fed according to USDA standards. Under this verification standard, grass or forage must be the exclusive feed source throughout the lifetime of the ruminant animal except for milk consumed before weaning. The animal cannot be fed grain or any grain byproduct before marketing and must have continuous access to pasture during the growing season. However, silage is an accepted feed that can consist of relatively large portions of grain. For example, corn silage, which averages 10 to 20% grain and can consist of up to a third or more grain, blurs the distinction between grain fed and forage fed (Mathews and Johnson, 2013).

In a survey of certified organic beef producers in the United States, 83% reported that cattle were raised exclusively or predominantly on grass and hay until slaughter, while the remaining 17% reported using a grain finishing system (Hafla et al., 2013). Organic beef cattle may be finished in feedlots for no more than 120 d and must have access to pasture during this time. In 2011, 106,181 beef cows (0.34% of the total U.S. beef cows; Table 2) and 113,114 unclassified cows and young stock were raised in certified organic production systems. The price of natural/organic beef averaged

$12.08/kg in the first quarter of 2011, which represented a premium of $3.75/kg.

Poultry

The largest volume of organic meat sales is for poultry. In 2011, the number of certified organic broilers totaled more than 28 million (0.33% of the total U.S. broilers; Table 2), layer hens totaled more than 6.6 million (1.97% of the total U.S. layers), and turkeys totaled 504,000 (0.20% of the total U.S. turkeys). In 2011, sales of U.S. organic broilers and eggs totaled $115 million and $276 million, representing 0.5 and 3.7% of total sales, respectively. The retail price for organic poultry and eggs between 2004 and 2006 was approximately twice that of conventional products (USDA Economic Research Service, 2012a).

Currently, the size of the market for products derived from animals raised in production systems that use either identity-preserved certified non-GE or organic feed is less than 5% (Fig. 7). Voluntary labeling programs and market premiums exist for products derived from animals that have not consumed GE feed. Mandating the labeling of products derived from animals that have eaten GE-feed at the current time would result in labeling essentially all products derived from conventionally raised livestock (i.e., >95% of all animal products) in the United States.

If suppliers and marketers respond to mandatory labeling of products from animals fed GE feed by increasing the offering of products from animals fed non-GE feed, an increase in the non-GE feed supply would be required. This could come from non-GE feed sources (e.g., wheat and barley), from contracting with U.S. growers to plant non-GE crop varieties, or from imported feed sources. Reversion from GE to conventional crop varieties would require the adoption of altered agronomic practices to manage those crops and relinquishment of the documented environmental and economic benefits associated with the adoption of GE crops (Areal et al., 2013; Fernandez-Cornejo et al., 2014; Green, 2012; NRC, 2010). The prices received by U.S. non-GE corn and soybean producers in recent years have averaged 15% more than the prices received by conventional commodity producers (CAST, 2014), and globally traded non-GE soybean meal is roughly at a 13% premium to conventional soybean meal prices. Given the importance of feed costs in overall animal production costs, the cost of animal products from animals fed non-GE feed would be more expensive.

Impact of Genetically Engineered Feedstuffs on the Sustainability of Livestock Production

Feedstuffs are a major contributor to life cycle assessments in the production of meat, milk, and eggs on a national and global scale. By 2020, developing countries will consume 107 million t more meat and 177 million t more milk than the annual average of the years 1996 through 1998. The projected increase in livestock production will require annual feed consumption of cereals to rise by nearly 300 million t by 2020 (Delgado, 2003). Despite the fact that the first generation of GE crops with so-called “input” traits (those that potentially alter inputs needed in production) were not designed to increase crops yields per se, GE technology has added an estimated 122 and 230 million t to the global production of soybeans and corn, respectively, since the introduction of GE varieties in the mid 1990s (Brookes and Barfoot, 2014a).

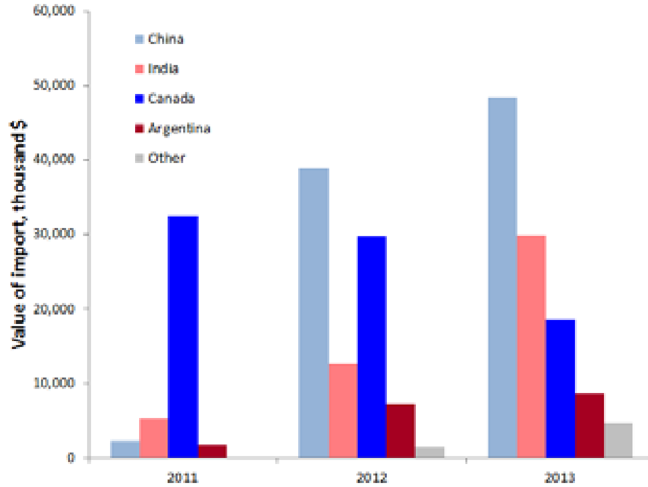

Figure 8. Value of certified National Organic Program soybeans imported into the United States, 2011 through 2013. Source: United States Department of Agriculture Foreign Agricultural Service (2014a). See online version for figure in color.

In 2013, approximately 175.2 million ha of GE crops were cultivated worldwide (James, 2013) by 18 million farmers. Over 90% (>16.5 million) were small-scale, resource-poor farmers in developing countries. This planting was greater than a 100-fold increase from the 1.7 million ha that were planted in 1996, making GE the fastest-adopted crop technology in recent history. India cultivated 11.0 million ha of Bt cotton with an adoption rate of 95%. In China, 7.5 million farmers cultivating an average of approximately 0.5 ha collectively grew 4.2 million ha of Bt cotton, an adoption rate of 90%. Farmers have planted these GE varieties to enable the adoption of improved agronomic practices (e.g., reduced insecticide applications) providing environmental, economic, and food security benefits in various countries (Ali and Abdulai, 2010; Burachik, 2010; Fernandez-Cornejo et al., 2014; Huang et al., 2010; Kathage and Qaim, 2012; Qaim and Kouser, 2013).

During the period 1996 through 2012, it has been estimated that the cumulative economic benefits from cost savings and added income derived from planting GE crops was $58.15 billion in developing countries and $58.45 billion in industrial countries (Brookes and

Barfoot, 2014a). The adoption of the technology also reduced pesticide spraying by 499 million kg (–8.7%), and has decreased the environmental impact of these crops by 18.1% (as measured by the indicator the Environmental Impact Quotient [a method that measures the environmental impact of pesticides]; Kovach et al., 1992) as a result of the use of less-toxic herbicides and reduced insecticide use (Brookes and Barfoot, 2014b). As a result of fuel savings associated with making fewer spray runs, the adoption of production systems with reduced tillage, and additional soil carbon sequestration, GE crops have also resulted in a significant reduction in the release of greenhouse gas emissions, which, in 2012 alone, was equivalent to removing 11.88 million cars from the roads (Brookes and Barfoot, 2014b).

Although some weed resistance has developed as a result of poor pest management practices and overreliance on a single herbicide (i.e., glyphosate), which may impact future benefits, the adoption of GE technology by the major livestock feed producing countries over the past 16 yr has had a positive sustainability outcome both in terms of increased global yield as a result of improved pest control and reduced overall environmental impacts per kilogram of animal feed produced.

The Future

There are numerous GE crops enhanced for animal nutrition in the research and development pipeline, with almost 100 events under research in many countries of the world (Tillie et al., 2013). This reflects both the importance of feed markets for GE crops and the potential nutritional improvements that can be brought to the quality of feedstuffs using this technology. There are 2 ways in which plant breeding might increase the efficiency of livestock production; the first is by raising the crop yield per hectare (e.g., improved drought tolerance or N use efficiency) and the second is by improving the rate of conversion of vegetable calories into animal calories (e.g., altered output traits or crop composition). Genetic engineering offers new possibilities for approaching both of these objectives, including improving the nutritional value of feed (e.g., AA content; Huang et al., 2006), lowering N and P pollution through altered crop composition (e.g., low phytate; Chen et al., 2008), and reducing manure excretion through a higher NE value (e.g., reduced lignin; Jung et al., 2012). Several of these crops are far advanced in the regulatory pipeline (Table 8; Tillie et al., 2013)

These so-called "second generation" crops modified for output traits will pose some regulatory and commercialization challenges. The first is that they will not, by definition, be substantially equivalent to isogenic non-GE varieties. Protocols have been developed to address the safety testing of these crops (International Life Sciences Institute, 2007). However, given the different regulatory approaches that are in place for crops that are compositionally equivalent, it is unclear how regulatory requirements may vary between countries in terms of the number and length of target animal feeding studies for these crops with altered output traits. Additionally, if the benefits derived from growing these crops accrue to the livestock producer or feeder and not directly to the farmer growing the crop, there will need to be some form of supply chain segregation in place to ensure a price premium is obtained for the value-added output trait.

An additional concern is the increasing problem of asynchronous regulatory approval, or regulatory asynchronicity. Currently, 33 countries have regulatory systems that handle approval for the cultivation or importation of new GE crops (International Service for the Acquisition of Agri-Biotech Applications, 2014). There are considerable discrepancies in the amount of time required to review and approve new GE crops in different countries. This leads to a situation where GE crops may be cultivated and marketed in some countries and remain unapproved in others. As discussed previously, this has resulted in trade disruptions, especially when countries use a "zero-tolerance" policy for unapproved events, meaning that even minute traces of unapproved GE crops are illegal and must be withdrawn from the market. Under a zero-tolerance policy, trade of relevant commodities between asynchronous countries will likely cease as importing and exporting firms will act to avoid the risk associated with a positive test (Kalaitzandonakes et al., 2014). Countries with zero-tolerance policies will be perceived as risky export markets, and importers will pay higher prices and insurance premiums to offset risks taken by the supplier.

Currently, the most accepted techniques for the detection of rDNA and protein products are PCR and ELISA, respectively. Various analytical methods have been developed and are routinely used for the monitoring of GE origin in raw materials and processed foods and have been reviewed elsewhere (Alexander et al., 2007; Marmiroli et al., 2008). Although efforts have been taken to harmonize analytical methodology for the detection of GE products at national, regional, and international levels, no international standards have yet been established (Holst-Jensen et al., 2006). Sampling, testing, and certification depend on statistical processes, however, and hence all are subject to some error, which increases at very low tolerances (Lamb and Booker, 2011).

Kalaitzandonakes et al. (2014) succinctly summarizes some emerging trends in terms of likely increased regulatory asynchronicity in the future. These include 1) the expanding pipeline of novel GE crop events, including second generation crops modified for output traits; 2) the expanding range of GE crop species being grown and

Table 8. Summary of genetically engineered crops modified for output traits in the latest stages of the pipeline. Modified from Tillie et al. (2013).

Crop	Identifier	Stage[1]	Commercial name	Trait	Developer[2]	Regulatory approval status					
						United States	Argentina	Brazil	China	European Union	Japan
Soybean	DP-305423-1	1	Treus-Plenish	High oleic acid	Pioneer	All uses – 2009	None	None	Food and feed – 2011 (expires 2014)	Food and feed application; additional data request – 2012	All uses – 2010
Safflower		1	Sonova 400	Omega-6	Arcadia BioSciences	Grown under permit; dietary supplement	None	None	None	None	None
Corn	BVLA430101	2		Phytase expression	CAAS/Originally in Agritech	None	None	None	None	None	Cultivation – 2009
Corn	REN-00038-3	2	Mavera	High lysine	Monsanto	All uses – 2006	None	None	None	Application withdrawn – 2009	All uses – 2007
Corn	REN-00038-3 × MON00810-6	2	Mavera YieldGard	High lysine + herbicide tolerance	Monsanto	All uses – 2006	None	None	None	Application withdrawn – 2009	All uses – 2007
Soybean	DP-305423-1 × MON04032-6	2		High oleic acid + herbicide tolerance	Pioneer	All uses – 2009	None	None	None	Food and feed application; additional data request – 2012	All uses – 2012
Soybean	MON-87705-6	2	Vistive Gold	High oleic acid	Monsanto	All uses – 2011	None	None	None	Imports and domestic use – 2012	Food and feed – 2013
Soybean[3]	DD-026005-3	2		High oleic acid	Pioneer	All uses – 1997	None	None	None	None	All uses – 2007
Alfalfa	MON-00179-5	3	None	Low lignin	Forage Genetics/ Monsanto	Food and feed – 2013	None	None	None	None	None
Rapeseed	MPS961-5	3	PhytaSeed	Phytase expression	BASF	Food and feed – 1999	None	None	None	None	None
Soybean	MON87769	3	None	Omega-3	Monsanto	All uses– 2011/2012	None	None	None	Food and feed application; additional data request – 2012	None

[1]Development stage: 1 = commercialized; 2 = commercial pipeline; 3 = regulatory pipeline.

[2]Pioneer, Johnston, IA; Arcadia Biosciences, Davis, CA; CAAS, Beijing, China; Monsanto, St. Louis, MO; Forage Genetics, Nampa, ID; BASF, Ludwigshafen, Germany.

[3]Events whose development is currently discontinued. The information regarding the regulatory status of the events reported in this table was updated in May 2014.

traded; 3) the expanding global hectarage of GE crops and the growing number of countries that raise them; and 4) the nascent and inexperienced regulatory expertise in many countries that will be called on to manage a large number of regulatory submissions for new GE crops in the future. Given the scope of trade of livestock feedstuffs and the increasing importance of GE crops in this supply, trade disruptions appear imminent, especially in countries that have slow approval processes for GE imports and yet are heavily dependent on commodity imports from exporting countries that are cultivating and developing a large number of GE crop varieties.

The emergence of precise gene-editing technologies (e.g., zinc finger nucleases [ZFN], meganucleases, transcription activator-like effector nucleases [TALEN], oligonucleotide-directed mutagenesis, and clustered regulatory interspaced short palindromic repeat [CRISPR]/Cas-based RNA-guided DNA endonucleases) that enable targeted editing of specific nucleotides in the endogenous genome (Kim and Kim, 2014) will further complicate this situation. Gene editing could be considered a form of directed mutagenesis and it is unclear whether gene-editing technologies for crops and animals will be encompassed by the GE regulatory system. This is especially uncertain where gene editing results in the substitution of 1 naturally occurring allelic form of a gene for another of the same gene or induces a mutation in an existing gene through a single base pair change analogous to the spontaneous mutation process (Wells, 2013). Whether these types of modifications should be subject to regulation is a topic of discussion among the global regulatory community (Bruce et al., 2013; Hartung and Schiemann, 2014; Lusser and Davies, 2013). Given that the regulatory process takes years and costs millions of dollars (Prado et al., 2014), the governance of emerging gene-editing technologies will have a great influence on the future development of crops carrying these genetic modifications and will significantly impact the ability of the public sector and small companies to bring gene-edited products to market.

Of particular practical importance is that there will be no way to differentiate a gene-edited DNA alteration from a naturally occurring mutation and hence no way to trace and track "genetically modified" gene-edited crops or differentiate them from genetic modifications resulting from spontaneous mutations. Many of the existing PCR-based tests for GE crops are designed using primers that amplify unique DNA sequences that are common to a variety of transgenic crops (e.g., exogenous promoter sequence or gene coding sequence). As new GE crops with multiple novel regulatory and coding region sequences are developed, it will be increasingly difficult to use PCR-based assays to detect all possible events. Furthermore, PCR-based screening methodology may be unable to detect the genetic modifications that are under development through precise breeding techniques (Lusser et al., 2012). Likewise, some gene-editing techniques generate genetic changes that cannot be distinguished from conventionally bred crops or from crops produced by natural genetic variation or unregulated radiation mutagenesis (Broeders et al., 2012). Process-based regulatory frameworks that rely on PCR-based detection of specific transgenic constructs will be unable keep pace with technological developments when the products of these advanced breeding techniques are indistinguishable from those produced using conventional breeding techniques.

These developments may lead to a revaluation of the current rDNA process-based regulatory trigger for GE organisms to a more scientifically defensible product-based approach centered on the novelty and any unique risks associated with the phenotype of the product rather than the process used to accomplish the genetic modification (Bradford et al., 2005; McHughen, 2007). The need for international coordination and synchronization of regulatory frameworks for GE products is becoming increasingly urgent as both research and development of GE crops and animals are proceeding at an accelerated rate in an ever increasing number of countries in the world. In the absence of international harmonization, costly trade disruptions are likely to become increasingly widespread in the future to the detriment of global food security.

Conclusions

Commercial livestock populations are the largest consumers of GE crops, and globally, billions of animals have been eating GE feed for almost 2 decades. An extensive search of peer-reviewed literature and field observations of animals fed diets containing GE crop products have revealed no unexpected perturbations or disturbing trends in animal performance or health indicators. Likewise, it is not possible to distinguish any differences in the nutritional profile of animal products following consumption of GE feed. Animal agriculture is currently highly dependent on GE feed sources, and global trade of livestock feed is largely supplied by countries that have approved the cultivation of GE crops. Supplying non-GE-fed animal products is likely to become increasingly expensive given the expanding global planting of GE crops and the growing number of countries that raise them. The market for animals that have not consumed GE feed is currently a niche market in the United States, although such products are available to interested consumers via voluntary process-based marketing programs. The cost of these products is higher than conventionally produced products due to both the higher cost of non-GE feed and the costs associated with certifying the absence of GE crops in the production process and product segregation. There is currently a pipeline of so-called "second generation" GE crops with improved output traits for livestock production. Their approval will further complicate the sourcing of non-GE feedstuffs. Additionally, recent developments in techniques to induce precise genetic changes in targeted genes offer both tremendous opportunities and a challenge for global regulatory oversight. Given these developments, there is an urgent need for international harmonization of both regulatory frameworks for GE crops and governance of advanced breeding techniques to prevent widespread disruptions in international trade of livestock feedstuffs in the future.

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UCDAVIS News and Information

No sign of health or nutrition problems from GMO livestock feed, study finds

September 25, 2014

ONE CALIFORNIA
One UCDAVIS

A new scientific review from the University of California, Davis, reports that the performance and health of food-producing animals consuming genetically engineered feed, first introduced 18 years ago, has been comparable to that of animals consuming non-GE feed.

The review study also found that scientific studies have detected no differences in the nutritional makeup of the meat, milk or other food products derived from animals that ate genetically engineered feed.

The review, led by UC Davis animal scientist Alison Van Eenennaam, examined nearly 30 years of livestock-feeding studies that represent more than 100 billion animals.

Titled "Prevalence and Impacts of Genetically Engineered Feedstuffs on Livestock Populations," the review article is now available online in open-access form through the American Society of Animal Science: https://asas.org/docs/default-source/jas-files/jas8124_final.pdf?sfvrsn. It will appear in print and open-access in the October issue of the Journal of Animal Science.

Genetically engineered crops were first introduced in 1996. Today, 19 genetically engineered plant species are approved for use in the United States, including the major crops used extensively in animal feed: alfalfa, canola, corn, cotton, soybean and sugar beet.

Food-producing animals such as cows, pigs, goats, chickens and other poultry species now consume 70 to 90 percent of all genetically engineered crops, according to the new UC Davis review. In the United States, alone, 9 billion food-producing animals are produced annually, with 95 percent of them consuming feed that contains genetically engineered ingredients.

"Studies have continually shown that the milk, meat and eggs derived from animals that have consumed GE feed are indistinguishable from the products derived from animals fed a non-GE diet," Van Eenennaam said. "Therefore, proposed labeling of animal products from livestock and poultry that have eaten GE feed would require supply-chain segregation and traceability, as the products themselves would not differ in any way that could be detected."

Now that a second generation of genetically engineered crops that have been optimized for livestock feed is on the horizon, there is a pressing need to internationally harmonize the regulatory framework for these products, she said.

"To avoid international trade disruptions, it is critical that the regulatory approval process for genetically engineered products be established in countries importing these feeds at the same time that regulatory approvals are passed in the countries that are major exporters of animal feed," Van Eenennaam said.

Collaborating on the study was co-author Amy E. Young in the UC Davis Department of Animal Science.

The review study was supported by funds from the W.K. Kellogg endowment and the California Agricultural Experiment Station of UC Davis.

UC Davis is growing California

At UC Davis, we and our partners are nourishing our state with food, economic activity and better health, playing a key part in the state's role as the top national agricultural producer for more than 50 years. UC Davis is participating in UC's Global Food Initiative launched by UC President Janet Napolitano, harnessing the collective power of UC to help feed the world and steer it on the path to sustainability.

About UC Davis

UC Davis is a global community of individuals united to better humanity and our natural world while seeking solutions to some of our most pressing challenges. Located near the California state capital, UC Davis has more than 34,000 students, and the full-time equivalent of 4,100 faculty and other academics and 17,400 staff. The campus has an annual research budget of over $750 million, a comprehensive health system and about two dozen specialized research centers. The university offers interdisciplinary graduate study and 99 undergraduate majors in four colleges and six professional schools.

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USDA: Genetically Modified Wheat Found in Montana

USDA says unapproved genetically modified wheat is found again, this time in Huntley, Montana

By MARY CLARE

The Associated Press

WASHINGTON

Unregulated genetically modified wheat has popped up in a second location in the United States, this time in Montana, the Agriculture Department said Friday.

No genetically engineered wheat has been approved for U.S. farming, and the discovery of unapproved varieties can pose a potential threat to U.S. trade with countries that have concerns about genetically modified foods.

USDA said Friday that the incident is on a smaller scale than a similar finding in Oregon last year that prompted several Asian countries to temporarily ban U.S. wheat imports.

The herbicide-resistant wheat was found on one to three acres in Montana, while the genetically engineered plants found in Oregon were spread over more than 100 acres. And the plants were found at a university research center in Huntley, Montana, where genetically modified wheat was legally tested by seed giant Monsanto 11 years ago. The plants in Oregon were found in a field that had never conducted such tests, prompting questions about how they got there.

The department said it is investigating the discovery of the Montana wheat, which is a different variety than the genetically modified wheat found in Oregon. USDA said the wheat would be safe to eat, but none of it entered the market.

In a final report also released Friday, USDA said it believes the genetically modified wheat in Oregon was an isolated incident and that there is no evidence of that wheat in commerce. The report says the government still doesn't know how the modified seeds got into the fields.

The discovery of the genetically modified wheat in Oregon in 2013 prompted Japan and South Korea to temporarily suspend some wheat orders, and the European Union called for more rigorous testing of U.S. shipments.

Monsanto Co. suggested last year that some of the company's detractors may have intentionally planted the seeds. Robb Fraley, Monsanto's executive vice president and chief technology officer, said in June 2013 that sabotage is the most likely scenario, partly because the modified wheat was not distributed evenly throughout the field and was found in patches.

"It's fair to say there are folks who don't like biotechnology and would use this to create problems," he said then.

Bernadette Juarez, who oversees investigative and enforcement efforts for USDA's Animal and Plant Health Inspection Service, said the department wasn't able to prove any such scenarios.

"Ultimately, we weren't able to make a determination of how it happened," she said.

In a statement Friday, a Monsanto spokeswoman did not repeat Fraley's 2013 speculation about sabotage but said the report provides closure. Monsanto also said it is fully cooperating with the investigation into the Montana wheat.

Montana State University's Southern Agricultural Research Center, where the modified wheat was found, also said it has been cooperating with USDA's investigation.

Most of the corn and soybeans grown in the United States are already genetically modified to resist certain herbicides or to have other traits. But the country's wheat crop is not, as some wheat farmers have shown reluctance to use genetically engineered seeds since their product is usually consumed directly by people. Much of the corn and soybean crop is used as feed for animals.

Some in the wheat industry have also been concerned that genetically modified wheat, if ever approved, would contaminate conventional wheat, causing problems with exports. Opponents of modified crops used the Oregon wheat as an example of that threat. "Genetic contamination is a serious threat to farmers across the country," said Andrew Kimbrell, executive director for Center for Food Safety.

There has been little evidence to show that foods grown from engineered seeds are less safe than their conventional counterparts, but several states have considered laws that would require them to be labeled so consumers know what they are eating. Vermont became the first state to enact such a law this year, though it is being challenged in court.

Follow Mary Clare Jalonick on Twitter at http://twitter.com/mcjalonick

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Pesticide Exposure and Depression among Male Private Pesticide Applicators in the Agricultural Health Study

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Background: Pesticide exposure may be positively associated with depression. Few previous studies have considered the episodic nature of depression or examined individual pesticides.

Objective: We evaluated associations between pesticide exposure and depression among male private pesticide applicators in the Agricultural Health Study.

Methods: We analyzed data for 10 pesticide classes and 50 specific pesticides used by 21,208 applicators enrolled in 1993–1997 who completed a follow-up telephone interview in 2005–2010. We divided applicators who reported a physician diagnosis of depression (*n* = 1,702; 8%) into those who reported a previous diagnosis of depression at enrollment but not follow-up (*n* = 474; 28%), at both enrollment and follow-up (*n* = 540; 32%), and at follow-up but not enrollment (*n* = 688; 40%) and used polytomous logistic regression to estimate odds ratios (ORs) and 95% CIs. We used inverse probability weighting to adjust for potential confounders and to account for the exclusion of 3,315 applicators with missing covariate data and 24,619 who did not complete the follow-up interview.

Results: After weighting for potential confounders, missing covariate data, and dropout, ever-use of two pesticide classes, fumigants and organochlorine insecticides, and seven individual pesticides—the fumigants aluminum phosphide and ethylene dibromide; the phenoxy herbicide (2,4,5-trichlorophenoxy)acetic acid (2,4,5-T); the organochlorine insecticide dieldrin; and the organophosphate insecticides diazinon, malathion, and parathion—were all positively associated with depression in each case group, with ORs between 1.1 and 1.9.

Conclusions: Our study supports a positive association between pesticide exposure and depression, including associations with several specific pesticides.

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Introduction

Exposure to pesticides, particularly organophosphate insecticides (OPs), may be positively associated with depression (Bazylewicz-Walczak et al. 1999; Beseler and Stallones 2008; Beseler et al. 2006, 2008; Mackenzie Ross et al. 2010; Onwuameze et al. 2013; Rehner et al. 2000; Salvi et al. 2003; Weisskopf et al. 2013; Wesseling et al. 2010). However, only a few of these studies were longitudinal (Bazylewicz-Walczak et al. 1999; Beseler and Stallones 2008; Onwuameze et al. 2013; Salvi et al. 2003)—an important consideration because many people with depression will recover and some may relapse (Colman and Ataullahjan 2010). The largest longitudinal study previously conducted (651 Colorado farmers and their spouses) assessed depression annually for three years using the Center for Epidemiological Studies-Depression Scale (CES-D) and found that individuals who reported past pesticide poisoning at baseline were twice as likely to be depressed during follow-up as those who did not (Beseler and Stallones 2008). That study, however, did not evaluate associations with chronic exposure in the absence of poisoning or to specific pesticides.

The Agricultural Health Study (AHS) is a prospective cohort study, including 52,394 licensed private pesticide applicators (mostly farmers), designed to assess associations between agricultural exposures and health end points (Alavanja et al. 1996). We previously found a higher prevalence of depression among male applicators who reported past pesticide poisoning or use of pesticides from several different classes (Beseler et al. 2008). That study, however, used a cross-sectional design and did not examine specific pesticides. The aim of the current study is to assess associations between pesticide use and depression among male pesticide applicators in the AHS.

Methods

Study population and case definition. From 1993 through 1997, pesticide applicators applying for or renewing their pesticide-use licenses at agricultural extension offices in Iowa and North Carolina were invited to enroll in the AHS (Alavanja et al. 1996). A total of 52,394 private applicators (84% of those eligible) enrolled by returning the enrollment questionnaire. An additional baseline questionnaire, the farmer questionnaire, was sent home with all enrolled applicators but returned by only 22,916 (44%). Applicators who returned the farmer questionnaire were older than those who did not, but generally similar otherwise (Tarone et al. 1997). A follow-up telephone interview in 2005–2010, an average of 12.1 years after enrollment, included questions on depression.

We excluded 6,567 applicators because they were female (1,358; 3%), were missing data on depression at enrollment and follow-up (1,894; 4%), or were missing covariate data (3,315; 6%); 45,827 (87%) applicators remained (Figure 1). In addition, 3,979 (8%) died before the follow-up interview and 20,640 (39%) did not complete it for other reasons. In total, we included 21,208 (40%) applicators in this analysis: 1,702 (8%) who reported ever receiving a physician's diagnosis

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M.R. is employed by Westat, Inc. (Durham, North Carolina), an employee-owned company. The authors declare they have no actual or potential competing financial interests.

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of depression (cases) and 19,506 (92%) who did not (noncases) (Figure 1).

Information on physician-diagnosed depression came from the enrollment and farmer questionnaires and the follow-up interview (AHS 2013). The enrollment questionnaire asked "Has a *doctor* ever told you that you had…[d]epression[?]" and the farmer questionnaire asked "Has a *DOCTOR* ever told you that you had (been diagnosed with)…[d]epression requiring medication or shock therapy?" We considered an applicator who responded affirmatively to either question to have a history of depression at enrollment. At follow-up, we asked "Have you ever been diagnosed with depression?" and "How old were you when you were first diagnosed with depression?" We considered any applicator who reported an age at diagnosis less than his age at enrollment to have a history of depression at enrollment regardless of his response to the enrollment depression questions.

We divided cases into three groups based on when the physician diagnosis of depression occurred (before or after enrollment) and on when it was reported via the AHS contacts (at enrollment, at follow-up, or both). The "pre-enrollment enrollment only" (PRE-E) group included 474 (28%) applicators who reported a previous diagnosis of depression at enrollment, but who did not confirm their pre-enrollment diagnosis at follow-up. The "pre-enrollment both" (PRE-B) group included 540 (32%) applicators who reported a previous diagnosis of depression at both enrollment and follow-up (n = 395), or who reported a previous diagnosis at follow-up only but with an age at diagnosis less than their age at enrollment (n = 145). The "post-enrollment" (POST) group included 688 (40%) applicators who reported a previous diagnosis of depression at follow-up but not at enrollment, and whose reported age at diagnosis equaled or exceeded their age at enrollment. Although both the PRE-E and PRE-B groups reported a diagnosis before enrollment, we treated them as separate outcomes in our analysis because we thought that the PRE-B group might be more likely to include men who had chronic depression, thus making them more likely to report a previous diagnosis at both time points, whereas the PRE-E group might not have reported a pre-enrollment diagnosis at follow-up because they did not experience depression during the follow-up period (12.1 years, on average). In addition, associations with pesticide use differed between the two groups. We cannot, however, confirm that the prevalence of depression over time differed between the two groups. It is also possible that PRE-E cases may have been less inclined to confirm their previous diagnosis of depression at follow-up because the interview was conducted via telephone, whereas depression information was collected at enrollment via self-administered paper questionnaires.

Some information on pesticide exposure was available only from the farmer questionnaire. Of the 21,208 applicators included in the analyses, 11,982 completed the farmer questionnaire. Of these, we classified 10,990 as noncases and 306 as PRE-E, 315 as PRE-B, and 371 as POST depression cases.

The AHS was approved by the institutional review boards (IRBs) of the National Institutes of Health and its contractors. The current analysis using coded data was exempted from review by the IRB of the University of North Carolina at Chapel Hill. All participants implied informed consent by returning the enrollment questionnaires and participating in the telephone interview.

Exposure assessment. At enrollment, applicators provided information on demographics, medical conditions, lifestyle, and pesticide use up until the time of enrollment by completing self-administered questionnaires (AHS 2013; Alavanja et al. 1996). We used three types of pesticide exposure variables: *a*) general exposure, *b*) use (personally mixed or applied) of pesticide classes, and *c*) use of individual pesticides. General exposure consisted of three variables: cumulative days of use of any pesticide, physician-diagnosed pesticide poisoning, and experiencing an incident of unusually high personal pesticide exposure (high pesticide exposure event). The latter two variables were available only for applicators who completed the farmer questionnaire. We calculated cumulative days of use of any pesticide as the product of reported duration (years) and frequency (days per year) and then categorized the result into four groups based on quartiles of use among all applicators. We created variables for ever-use of pesticides from four functional classes (fumigants, fungicides, herbicides, and insecticides) and six chemical classes (phenoxy and triazine herbicides, carbamates, and organochlorine, organophosphate, and pyrethroid insecticides) based on responses for individual pesticides. Use of 50 individual pesticides included ever-use and cumulative days of use. Information on ever-use was collected via the enrollment questionnaire for all 50 pesticides, whereas information on duration and frequency, used to calculate cumulative days of use, was collected via the enrollment

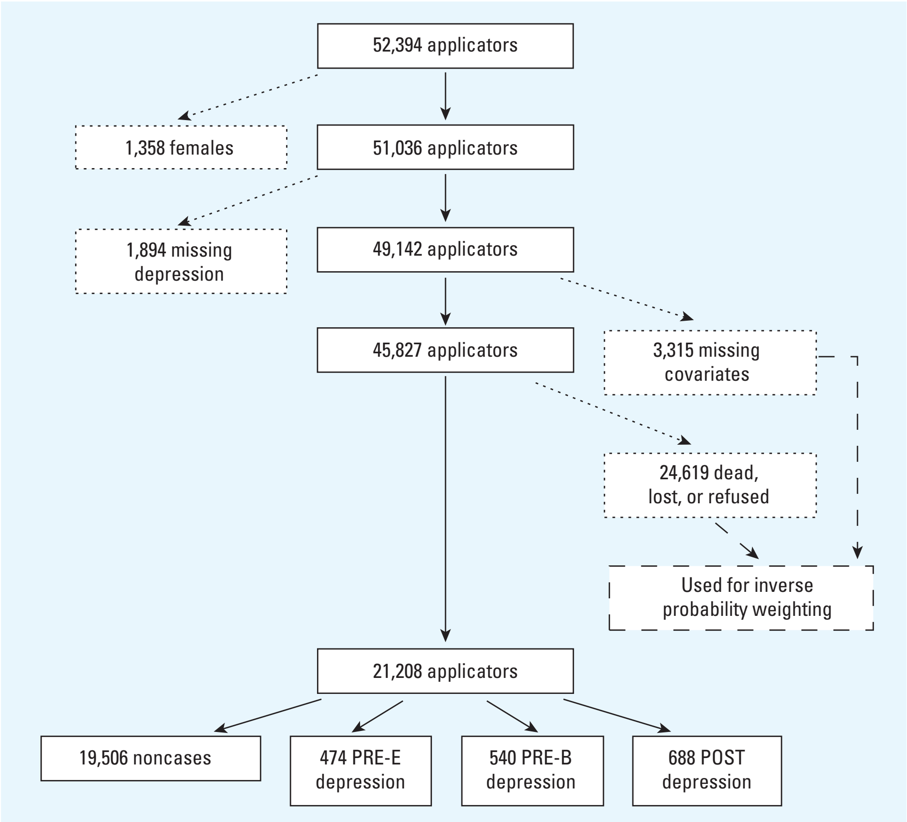

Figure 1. Flow diagram depicting the study population for an analysis of pesticide use and self-reported depression among male private pesticide applicators in the AHS. Solid boxes or lines represent individuals remaining in the study after each step; small-dashed boxes or lines represent individuals excluded after each step (see "Study population and case definition" for more details); large-dashed boxes or lines represent individuals incorporated into the analysis only indirectly via inverse probability weighting (see "Statistical analyses" for more details). Depression groups shown at the bottom of the diagram were defined as described in the text (see "Study population and case definition" for more details).

questionnaire for 22 pesticides and via the farmer questionnaire for the other 28. We calculated cumulative days of use for individual pesticides as the product of duration and frequency variables and then categorized the result into four groups: nonusers plus users categorized at tertiles. For six pesticides, at least two of the 12 exposure-category by depression-group combinations had fewer than five cases, so we instead used three groups: nonusers plus users dichotomized at the median.

Statistical analyses. We had information from the enrollment questionnaire on potential confounders identified from previous literature: age, state, education, marital status, number of children in family, usual frequency of alcohol consumption per week in the past year, cigarette smoking, diabetes (an indication of chronic disease), farm size, and wearing chemical-resistant gloves when personally handling pesticides. For applicators who completed the farmer questionnaire, we also had information on number of doctor visits in the past year (an indication of general health), number of years lived or worked on a farm, working a job off a farm, and solvent (other than gasoline) exposure in the longest-held nonfarm job.

We used a directed acyclic graph (Greenland et al. 1999) to identify two minimally sufficient adjustment sets (MSASs) among potential confounders: *a*) age, alcohol consumption, diabetes, marital status, smoking, solvents, and state; and *b*) age, diabetes, education, and state (see Supplemental Material, Figure S1). This report used the second MSAS because it had less missing covariate information; the first MSAS gave similar results (data not shown).

For our main analyses, we used stabilized inverse probability weights to adjust for confounding and to account for the loss of 3,315 applicators with missing covariate data (in diabetes and education) and 24,619 applicators who did not complete the follow-up interview (Cole and Hernán 2008). For analyses involving information from the farmer questionnaire, we added a weight to account for the loss of 9,226 applicators who did not complete that questionnaire. We used polytomous logistic regression to estimate odds ratios (ORs) and 95% CIs for associations between pesticide exposure and depression within each case group, using noncases as the reference. These ORs apply to the population of 49,142 male applicators not missing data on depression at enrollment and at follow-up. We rounded all ORs and 95% CIs to the tenths place for presentation, and considered pesticide exposure to be "positively associated" with depression if the rounded lower 95% confidence limit for the OR was at least 1.0 or if the rounded OR was at least 1.3. We used Wald chi-square tests to test differences among case group–specific ORs at $\alpha = 0.1$. We assessed linear trends for cumulative-days-of-use variables using the medians of each exposure category. We modeled the median category scores as continuous variables and scaled the trend ORs to interquartile range (IQR) increases in the original cumulative-days-of-use variables.

We used linear, logistic, or ordinal logistic regression, depending on the nature of the exposure variable, to calculate stabilized weights for confounding, missing covariate data, missing farmer questionnaire (if appropriate), and dropout for each exposure separately and then multiplied the three or four weights to obtain the overall stabilized weight (Cole and Hernán 2008; see also Supplemental Material, p. 4). In all models used to calculate the weights (see Supplemental Material, p. 4), we fit age as a restricted, quadratic spline with knots at 40, 48, and 57 years of age based on percentiles of the age distribution in all cases whereas diabetes, education, and state were modeled as shown in Table 1. We applied the overall stabilized weight to polytomous logistic regression models for depression that contained the exposure of interest as the only explanatory variable in the same way that sampling weights are applied when analyzing data from complex survey sampling designs (Cole and Hernán 2008). We calculated 95% CIs using robust variance estimates because using weights induces within-subject correlation (Hernán et al. 2000). We also conducted a sensitivity analysis without weighting; we used standard regression methods to adjust for potential confounding but without adjustment for potential biases from missing covariate data, missing farmer questionnaire, or dropout.

Table 1. Selected characteristics of male private pesticide applicators in the AHS.

Characteristic	Noncases [*n* (%)]	PRE-E[a] Cases [*n* (%)]	PRE-E[a] Adjusted OR[b] (95% CI)	PRE-B[a] Cases [*n* (%)]	PRE-B[a] Adjusted OR[b] (95% CI)	POST[a] Cases [*n* (%)]	POST[a] Adjusted OR[b] (95% CI)	*p* for difference among ORs[c]
Total	19,506 (100)	474 (100)		540 (100)		688 (100)		
Age at enrollment (years)								
≤ 25	540 (3)	5 (1)	0.4 (0.2, 1.0)	7 (1)	0.5 (0.2, 1.0)	9 (1)	0.4 (0.2, 0.8)	
26–35	2,879 (15)	25 (5)	0.4 (0.2, 0.6)	36 (7)	0.5 (0.3, 0.7)	119 (17)	1.0 (0.8, 1.3)	
36–45	5,856 (30)	136 (29)	Reference	158 (29)	Reference	238 (35)	Reference	
46–55	4,909 (25)	143 (30)	1.3 (1.0, 1.6)	177 (33)	1.3 (1.1, 1.7)	184 (27)	0.9 (0.7, 1.1)	
56–65	3,902 (20)	120 (25)	1.3 (1.1, 1.7)	118 (22)	1.1 (0.9, 1.4)	96 (14)	0.6 (0.5, 0.8)	
> 65	1,420 (7)	45 (9)	1.4 (1.0, 2.0)	44 (8)	1.2 (0.8, 1.7)	42 (6)	0.7 (0.5, 1.0)	< 0.01
State of residence								
Iowa	13,520 (69)	329 (69)	Reference	384 (71)	Reference	460 (67)	Reference	
North Carolina	5,986 (31)	145 (31)	0.9 (0.8, 1.1)	156 (29)	0.9 (0.7, 1.1)	228 (33)	1.2 (1.0, 1.4)	0.04
Education level								
≤ Some high school or something else	1,343 (7)	48 (10)	1.4 (1.0, 1.9)	44 (8)	1.2 (0.8, 1.6)	45 (7)	1.1 (0.8, 1.5)	
High school graduate or GED	9,045 (46)	213 (45)	Reference	251 (46)	Reference	314 (46)	Reference	
1–3 years of vocational education beyond high school, some college, or college graduate	8,357 (43)	192 (41)	1.1 (0.9, 1.3)	226 (42)	1.0 (0.9, 1.2)	297 (43)	1.0 (0.8, 1.1)	
≥ 1 years of graduate or professional school	761 (4)	21 (4)	1.1 (0.7, 1.7)	19 (4)	0.9 (0.5, 1.4)	32 (5)	1.2 (0.8, 1.7)	0.79
Ever diagnosed with diabetes								
No	19,051 (98)	450 (95)	Reference	516 (96)	Reference	665 (97)	Reference	
Yes	455 (2)	24 (5)	1.9 (1.2, 2.9)	24 (4)	1.8 (1.2, 2.7)	23 (3)	1.6 (1.0, 2.5)	0.84

Abbreviations: GED, General Equivalency Diploma; POST, post-enrollment; PRE-B, pre-enrollment both; PRE-E, pre-enrollment enrollment only.
[a]Cases were divided into three groups based on when the physician diagnosis of depression occurred (before or after enrollment) and on when it was reported via the AHS contacts (at enrollment, at follow-up, or both). The PRE-E group included applicators who reported a previous diagnosis of depression at enrollment, but who did not confirm their pre-enrollment diagnosis at follow-up. The PRE-B group included applicators who reported a previous diagnosis of depression at both enrollment and follow-up, or who reported a previous diagnosis at follow-up only but with an age at diagnosis less than their age at enrollment. The POST group included applicators who reported a previous diagnosis of depression at follow-up but not enrollment, and whose reported age at diagnosis equaled or exceeded their age at enrollment. [b]Adjusted for age at enrollment (modeled with a cubic polynomial) and state of residence. [c]Differences among case group–specific ORs tested via Wald chi-square tests.

We used four criteria to evaluate the appropriateness of the weights used in our analyses: *a*) nearness of the mean weight to one, *b*) number of extreme weights (e.g., < 0.05 or > 20), *c*) positivity, and *d*) bias–variance (validity–precision) tradeoff (Cole and Hernán 2008). We did not consider the c-statistic, Hosmer–Lemeshow statistic, or any other measure of goodness-of-fit to select variables for inclusion in our models for the weights because doing so can lead to bias (from unbalanced confounders or balanced nonconfounders including instrumental variables), reduced precision, nonpositivity, and/or restricted inference (Westreich et al. 2011). To informally assess the bias–variance tradeoff (Winer 1978), we progressively truncated the overall stabilized weights by resetting weights less (or greater) than a certain percentile to the value of that percentile (Cole and Hernán 2008). Regarding the ORs derived from the untruncated weights as the "true" values, we informally evaluated bias–variance tradeoff by evaluating how features of both the weights and the corresponding ORs changed with increasing truncation. We considered nearness of the mean weight to one, reduction in number of extreme weights, and a balance between increased "bias" and reduced variance in the estimated ORs (Cole and Hernán 2008). Truncating the overall stabilized weights at the first and 99th percentiles appeared to be the best balance of validity and precision and mitigated problems identified by all of the criteria in this analysis.

We conducted several additional sensitivity analyses. We augmented models for ever-use of pesticide classes or individual pesticides by adding potentially confounding variables one at a time in models for all the different types of weights. These variables were number of children, doctor visits in the past year, farm size, use of chemical-resistant gloves, and cumulative lifetime days of use of any pesticide. We included all variables in Table 1 and in Supplemental Material, Table S1, in models for the dropout weights to evaluate whether there were selection effects beyond that captured by the covariates in the second MSAS. To account for correlations between use of different pesticides, we added the pesticide that was most strongly correlated with the pesticide of interest to models for the weights. We refit models excluding applicators who reported physician-diagnosed pesticide poisoning to evaluate whether or not results were driven by pesticide poisoning. Finally, we evaluated effect measure modification by state or by use of chemical-resistant gloves using the likelihood ratio test at $\alpha = 0.1$. We performed all analyses via SAS version 9.2 (SAS Institute Inc., Cary, NC).

Results

After adjustment for age at enrollment and state of residence, the odds of depression were higher in each case group for applicators who were past cigarette smokers compared with those who never smoked, who reported at least one visit to a medical doctor in the past year compared with no visits, and who reported a previous diagnosis of diabetes compared with none (Table 1; see also Supplemental Material, Table S1). For age, state, marital status, doctor visits in the past year, and solvent (other than gasoline) exposure in the longest-held nonfarm job, ORs for POST depression were generally different from ORs for PRE-E and PRE-B depression, whereas the latter two were generally similar (Table 1; see also Supplemental Material, Table S1).

The mean weight of all truncated overall stabilized weights was approximately one except that for the categorical version of cumulative days of carbaryl use (mean weight = 1.28). There were no extreme weights (see Supplemental Material, Tables S2–S4).

After weighting for age, diabetes diagnosis, education, state, missing covariate data, missing farmer questionnaire (where appropriate), and dropout, depression was positively associated with cumulative days of use of any pesticide, physician-diagnosed pesticide poisoning, and ever experiencing a high pesticide exposure event among PRE-E and PRE-B cases, but not among POST cases (Table 2). In each case group, depression was positively associated with ever-use of fumigants as a class and organochlorine insecticides as a class as well as the specific fumigants aluminum phosphide and ethylene dibromide; the phenoxy herbicide (2,4,5-trichlorophenoxy)acetic acid (2,4,5-T); the organochlorine insecticide dieldrin; and the OPs diazinon, malathion, and parathion (Table 3).

Many pesticides were positively associated with depression in one or two, but not all three, case groups, but the ORs did not differ significantly (Table 3). Wald chi-square

Table 2. Pesticide use and self-reported depression among male private pesticide applicators in the AHS.

Variable	Noncases [*n* (%)]	PRE-E[a] Cases [*n* (%)]	PRE-E[a] IP-weighted OR[b] (95% CI)	PRE-B[a] Cases [*n* (%)]	PRE-B[a] IP-weighted OR[b] (95% CI)	POST[a] Cases [*n* (%)]	POST[a] IP-weighted OR[b] (95% CI)	*p* for difference among ORs[c]
Total	19,506 (100)	474 (100)		540 (100)		688 (100)		
Cumulative days personally mixed or applied pesticides[d]								
≤ 56 (median = 24.5)	4,520 (23)	79 (17)	Reference	102 (19)	Reference	164 (24)	Reference	
57–225 (median = 116.0)	6,876 (35)	164 (35)	1.2 (0.9, 1.6)	189 (35)	1.1 (0.8, 1.4)	223 (32)	0.9 (0.7, 1.1)	
226–457 (median = 369.8)	4,139 (21)	107 (23)	1.4 (1.0, 1.9)	129 (24)	1.3 (1.0, 1.8)	170 (25)	1.1 (0.9, 1.4)	
> 457 (median = 767.3)	3,968 (20)	124 (26)	1.6 (1.2, 2.2)	120 (22)	1.3 (1.1, 1.7)	131 (19)	0.9 (0.7, 1.2)	0.10
Missing	3	0		0		0		
Trend (IQR = 401.3)[e]			1.3 (1.1, 1.4)		1.1 (1.0, 1.3)		1.0 (0.9, 1.1)	0.03
Ever diagnosed with pesticide poisoning[f]								
No	10,656 (98)	274 (90)	Reference	293 (95)	Reference	362 (98)	Reference	
Yes	206 (2)	29 (10)	4.2 (2.7, 6.6)	16 (5)	2.5 (1.4, 4.4)	7 (2)	1.0 (0.4, 2.4)	0.01
Missing	128	3		6		2		
Ever experienced an incident of unusually high personal pesticide exposure[f]								
No	9,093 (85)	215 (72)	Reference	214 (71)	Reference	296 (83)	Reference	
Yes	1,642 (15)	84 (28)	2.3 (1.8, 3.1)	86 (29)	2.2 (1.6, 2.9)	60 (17)	1.1 (0.8, 1.5)	< 0.01
Missing	255	7		15		15		

Abbreviations: IP, inverse probability; POST, post-enrollment; PRE-B, pre-enrollment both; PRE-E, pre-enrollment enrollment only.
[a]See Table 1 for a description of the three case groups. [b]Weights were adjusted for age at enrollment (modeled with a restricted, quadratic spline with knots at 40, 48, and 57 years of age based on percentiles of the age distribution in cases), ever diagnosed with diabetes, education level, state of residence, not missing covariate data (conditional on age, state, the exposure, and pairwise interaction terms between each covariate and the exposure), and not dropping out of the AHS cohort (conditional on age, diabetes, education, state, the exposure, and pairwise interaction terms between each covariate and the exposure). 95% CIs were calculated with robust variance estimates. [c]Differences among case group–specific ORs were tested via Wald chi-square tests. [d]Category boundaries were set at quartiles of cumulative days of pesticide use among all male private pesticide applicators. [e]We used within-category medians and scaled the OR to an IQR-unit (days) increase in cumulative days of pesticide use among all male private pesticide applicators. [f]Data were available only for 11,982 applicators who completed the farmer questionnaire. Weights were additionally adjusted for completing the farmer questionnaire (conditional on age, diabetes, education, and state).

Table 3. Ever-use of pesticide classes and specific pesticides and self-reported depression among male private pesticide applicators in the AHS.

		PRE-E[b]		PRE-B[b]		POST[b]		
Ever personally mixed or applied	Noncases[a] [*n* (%)]	Cases[a] [*n* (%)]	IP-weighted OR[c,d] (95% CI)	Cases[a] [*n* (%)]	IP-weighted OR[c,d] (95% CI)	Cases[a] [*n* (%)]	IP-weighted OR[c,d] (95% CI)	*p* for difference among ORs[e]
Total	19,506 (100)	474 (100)		540 (100)		688 (100)		
Fumigants	4,363 (23)	131 (29)	1.4 (1.1, 1.8)	166 (32)	1.8 (1.5, 2.3)	177 (27)	1.2 (1.0, 1.5)	0.03
Aluminum phosphide	940 (5)	32 (7)	1.4 (0.9, 2.0)	38 (7)	1.3 (0.9, 1.9)	49 (8)	1.6 (1.1, 2.2)	0.75
Carbon tetrachloride/carbon disulfide (80/20 mix)	1,164 (6)	46 (10)	1.8 (1.3, 2.6)	53 (11)	1.9 (1.4, 2.7)	44 (7)	1.2 (0.8, 1.7)	0.11
Ethylene dibromide	676 (4)	24 (5)	1.7 (1.0, 2.7)	25 (5)	1.5 (1.0, 2.4)	29 (5)	1.3 (0.9, 2.1)	0.79
Methyl bromide	2,853 (15)	75 (16)	1.2 (0.7, 1.9)	90 (17)	1.6 (1.0, 2.4)	109 (16)	1.2 (0.8, 1.8)	0.57
Fungicides	6,850 (36)	184 (40)	1.2 (1.0, 1.5)	213 (41)	1.3 (1.1, 1.6)	256 (39)	1.1 (0.9, 1.3)	0.33
Benomyl[f]	1,793 (10)	50 (11)	1.5 (1.0, 2.2)	48 (9)	1.1 (0.7, 1.7)	70 (11)	1.3 (0.9, 1.8)	0.67
Captan	2,301 (12)	62 (14)	1.2 (0.9, 1.5)	86 (17)	1.4 (1.1, 1.8)	90 (14)	1.2 (0.9, 1.5)	0.52
Chlorothalonil	1,326 (7)	31 (7)	0.9 (0.5, 1.5)	43 (8)	1.3 (0.8, 2.0)	55 (8)	1.2 (0.8, 1.7)	0.58
Maneb/mancozeb	1,775 (10)	50 (11)	1.3 (0.8, 2.0)	51 (10)	1.2 (0.7, 1.8)	65 (10)	1.2 (0.8, 1.8)	0.95
Metalaxyl	4,157 (22)	120 (27)	1.5 (1.1, 1.9)	122 (24)	1.3 (1.0, 1.7)	151 (23)	1.0 (0.8, 1.3)	0.12
Ziram	276 (2)	10 (2)	1.6 (0.8, 3.1)	5 (1)	0.8 (0.3, 2.0)	12 (2)	1.4 (0.8, 2.6)	0.46
Herbicides	19,086 (98)	469 (99)	1.6 (0.7, 4.0)	533 (99)	1.8 (0.8, 3.9)	677 (99)	1.1 (0.6, 2.1)	0.62
Alachlor	10,526 (56)	287 (63)	1.3 (1.0, 1.6)	325 (62)	1.2 (1.0, 1.4)	384 (59)	1.1 (0.9, 1.3)	0.61
Butylate	6,338 (34)	162 (36)	1.1 (0.9, 1.3)	196 (39)	1.1 (0.9, 1.3)	234 (36)	1.1 (0.9, 1.3)	0.80
Chlorimuron-ethyl	7,077 (38)	160 (36)	0.9 (0.8, 1.2)	199 (39)	1.1 (0.9, 1.3)	261 (40)	1.0 (0.9, 1.2)	0.59
Dicamba	10,237 (55)	248 (54)	0.9 (0.7, 1.1)	292 (57)	1.0 (0.8, 1.2)	365 (57)	1.0 (0.8, 1.2)	0.74
EPTC	4,013 (22)	113 (25)	1.2 (0.9, 1.5)	105 (21)	0.9 (0.7, 1.2)	156 (24)	1.0 (0.8, 1.3)	0.44
Glyphosate	15,053 (78)	376 (80)	1.2 (0.9, 1.6)	426 (79)	1.1 (0.9, 1.4)	540 (79)	1.1 (0.9, 1.3)	0.80
Imazethapyr	8,480 (46)	207 (46)	1.0 (0.8, 1.3)	220 (43)	0.9 (0.7, 1.1)	304 (47)	1.1 (0.9, 1.3)	0.42
Metolachlor	9,121 (49)	229 (51)	1.1 (0.9, 1.3)	231 (45)	0.8 (0.7, 1.0)	311 (48)	1.0 (0.8, 1.1)	0.20
Paraquat	4,402 (24)	120 (26)	1.2 (1.0, 1.5)	123 (25)	1.1 (0.9, 1.4)	158 (24)	1.1 (0.9, 1.3)	0.77
Pendimethalin	8,372 (45)	218 (48)	1.2 (1.0, 1.4)	217 (42)	0.9 (0.8, 1.1)	282 (43)	0.9 (0.8, 1.1)	0.09
Petroleum oil	9,408 (51)	260 (58)	1.3 (1.1, 1.6)	285 (57)	1.2 (0.9, 1.5)	336 (52)	1.0 (0.9, 1.2)	0.11
Trifluralin	10,286 (55)	266 (59)	1.2 (1.0, 1.5)	299 (58)	1.1 (0.9, 1.3)	363 (56)	1.1 (0.9, 1.3)	0.63
Phenoxy herbicides	15,742 (82)	391 (84)	1.1 (0.9, 1.5)	456 (86)	1.3 (1.0, 1.7)	541 (80)	0.9 (0.8, 1.1)	0.11
2,4-D	15,371 (79)	378 (81)	1.1 (0.8, 1.4)	442 (82)	1.2 (0.9, 1.5)	526 (78)	1.0 (0.8, 1.2)	0.45
2,4,5-T	4,517 (24)	157 (35)	1.6 (1.3, 2.0)	178 (35)	1.6 (1.3, 1.9)	157 (24)	1.2 (1.0, 1.5)	0.10
2,4,5-TP	1,841 (10)	71 (16)	1.7 (1.3, 2.2)	73 (14)	1.7 (1.3, 2.2)	67 (11)	1.1 (0.9, 1.5)	0.07
Triazine herbicides	15,768 (82)	393 (84)	1.1 (0.8, 1.5)	445 (83)	1.0 (0.8, 1.3)	556 (82)	1.1 (0.8, 1.3)	0.91
Atrazine	14,554 (75)	372 (79)	1.2 (1.0, 1.6)	415 (77)	1.0 (0.8, 1.3)	511 (75)	1.0 (0.8, 1.2)	0.44
Cyanazine	8,399 (45)	233 (51)	1.3 (1.0, 1.6)	258 (50)	1.1 (0.9, 1.3)	304 (46)	1.1 (0.9, 1.4)	0.55
Metribuzin	9,061 (49)	236 (52)	1.1 (0.9, 1.4)	264 (52)	1.0 (0.9, 1.3)	322 (49)	1.0 (0.9, 1.2)	0.83
Insecticides	18,379 (95)	458 (97)	1.3 (0.7, 2.2)	510 (95)	1.0 (0.6, 1.5)	655 (97)	1.5 (1.0, 2.4)	0.34
Carbamates[f]	13,037 (68)	335 (71)	1.0 (0.8, 1.3)	389 (73)	1.0 (0.8, 1.3)	475 (70)	1.1 (0.9, 1.3)	0.95
Aldicarb	1,891 (10)	42 (9)	0.9 (0.6, 1.5)	52 (10)	1.4 (1.0, 2.2)	81 (13)	1.4 (1.0, 1.9)	0.28
Carbaryl	10,984 (58)	295 (64)	1.2 (0.9, 1.5)	336 (64)	1.2 (1.0, 1.5)	411 (62)	1.1 (0.9, 1.4)	0.87
Carbofuran	5,576 (30)	153 (34)	1.2 (1.0, 1.5)	181 (35)	1.2 (1.0, 1.5)	180 (28)	0.9 (0.8, 1.1)	0.14
Organochlorine insecticides	10,316 (55)	333 (72)	1.9 (1.5, 2.4)	334 (64)	1.2 (1.0, 1.4)	368 (56)	1.2 (1.0, 1.5)	0.01
Aldrin	3,991 (22)	140 (31)	1.4 (1.1, 1.9)	159 (31)	1.5 (1.2, 1.9)	137 (21)	1.2 (0.9, 1.5)	0.36
Chlordane	5,321 (28)	185 (41)	1.6 (1.3, 2.0)	179 (35)	1.3 (1.0, 1.6)	185 (29)	1.1 (0.9, 1.3)	0.03
DDT	5,152 (28)	174 (38)	1.8 (1.4, 2.3)	175 (34)	1.3 (1.0, 1.7)	143 (22)	1.0 (0.7, 1.3)	0.01
Dieldrin	1,476 (8)	56 (13)	1.6 (1.1, 2.3)	59 (12)	1.6 (1.1, 2.2)	48 (7)	1.3 (0.9, 1.8)	0.63
Heptachlor	3,354 (18)	131 (29)	1.6 (1.3, 2.1)	126 (25)	1.3 (1.0, 1.7)	100 (16)	1.0 (0.8, 1.3)	0.04
Lindane	4,053 (22)	146 (32)	1.6 (1.3, 2.0)	141 (28)	1.3 (1.0, 1.6)	152 (23)	1.2 (0.9, 1.4)	0.08
Toxaphene	2,899 (16)	97 (22)	1.5 (1.1, 1.9)	110 (22)	1.5 (1.2, 1.9)	104 (16)	1.1 (0.9, 1.4)	0.12
Organophosphate insecticides	17,563 (91)	442 (94)	1.6 (1.1, 2.3)	494 (92)	1.2 (0.8, 1.7)	629 (93)	1.3 (1.0, 1.8)	0.56
Chlorpyrifos	8,457 (44)	221 (47)	1.2 (1.0, 1.4)	272 (50)	1.3 (1.1, 1.5)	300 (44)	1.0 (0.9, 1.2)	0.10
Coumaphos	1,799 (10)	57 (13)	1.2 (0.9, 1.7)	63 (13)	1.3 (1.0, 1.7)	54 (9)	0.8 (0.6, 1.1)	0.03
Diazinon	6,211 (33)	182 (40)	1.4 (1.1, 1.7)	207 (41)	1.3 (1.1, 1.6)	235 (36)	1.2 (1.0, 1.4)	0.51
Dichlorvos	1,856 (12)	61 (14)	1.1 (0.8, 1.5)	96 (19)	1.6 (1.3, 2.1)	99 (15)	1.3 (1.0, 1.6)	0.11
Fonofos	4,396 (24)	132 (29)	1.3 (1.0, 1.7)	144 (28)	1.1 (0.9, 1.4)	146 (23)	0.9 (0.7, 1.2)	0.18
Malathion	13,941 (74)	369 (80)	1.3 (1.0, 1.7)	410 (79)	1.2 (1.0, 1.6)	503 (76)	1.1 (1.0, 1.4)	0.62
Parathion	2,903 (16)	102 (23)	1.5 (1.2, 1.9)	95 (19)	1.2 (1.0, 1.6)	116 (18)	1.3 (1.0, 1.6)	0.51
Phorate	6,523 (35)	191 (42)	1.3 (1.0, 1.6)	196 (38)	1.0 (0.8, 1.2)	228 (35)	1.0 (0.8, 1.2)	0.25
Terbufos	7,746 (42)	223 (50)	1.4 (1.1, 1.7)	240 (47)	1.2 (1.0, 1.4)	265 (41)	1.0 (0.8, 1.2)	0.07
Trichlorfon	123 (1)	5 (1)	1.5 (0.6, 3.7)	2 (1)	—[g]	1 (< 1)	—[g]	—[g]
Pyrethroid insecticides	4,805 (26)	128 (28)	1.2 (1.0, 1.5)	146 (28)	1.1 (0.9, 1.4)	164 (25)	0.9 (0.8, 1.1)	0.17
Permethrin (for animals)	2,841 (15)	78 (17)	1.2 (0.9, 1.5)	87 (17)	1.0 (0.8, 1.4)	104 (16)	1.0 (0.8, 1.3)	0.74
Permethrin (for crops)	2,539 (14)	68 (15)	1.2 (0.9, 1.6)	85 (17)	1.3 (1.0, 1.7)	82 (13)	0.9 (0.7, 1.2)	0.09

Abbreviations: 2,4-D, (2,4-dichlorophenoxy)acetic acid; 2,4,5-T, (2,4,5-trichlorophenoxy)acetic acid; 2,4,5-TP, (*RS*)-2-(2,4,5-trichlorophenoxy)propionic acid; DDT, 1,1,1-trichloro-2,2-bis(4-chlorophenyl) ethane; EPTC, *S*-ethyl dipropyl(thiocarbamate); IP, inverse probability; POST, post-enrollment; PRE-B, pre-enrollment both; PRE-E, pre-enrollment enrollment only.
[a]Information for specific pesticides was missing for < 1–6% of male private pesticide applicators. [b]See Table 1 for a description of the three case groups. [c]Male private pesticide applicators who did not use each pesticide class or specific pesticide were the reference. [d]Weights were adjusted for age at enrollment (modeled with a restricted, quadratic spline with knots at 40, 48, and 57 years of age based on percentiles of the age distribution in cases), ever diagnosed with diabetes, education level, state of residence, not missing covariate data (conditional on age, state, the exposure, and pairwise interaction terms between each covariate and the exposure), and not dropping out of the AHS cohort (conditional on age, diabetes, education, state, the exposure, and pairwise interaction terms between each covariate and the exposure). 95% CIs were calculated with robust variance estimates. [e]Differences among case group–specific ORs were tested via Wald chi-square tests. [f]Benomyl is also included in carbamates. [g]OR (95% CI) and *p* for difference not shown because fewer than five PRE-B or POST cases ever personally mixed or applied trichlorfon.

tests indicated that associations for ever-use of two pesticide classes and nine specific pesticides differed significantly at $\alpha = 0.1$ among case groups. ORs for PRE-B depression were higher than those for PRE-E and POST depression for fumigants as a class, whereas ORs for PRE-E depression were higher than those for PRE-B and POST depression for organochlorine insecticides as a class (Table 3). For the nine specific pesticides, the most consistent finding was that ORs were elevated (lower 95% confidence limit ≥ 1.0 or OR ≥ 1.3) for PRE-E and PRE-B depression, but not for POST depression; this pattern was observed for the phenoxy herbicide (*RS*)-2-(2,4,5-trichlorophenoxy)propionic acid (2,4,5-TP); the organochlorine insecticides chlordane, 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane (DDT), heptachlor, and lindane; and the OP terbufos (Table 3).

We observed positive trend ORs, based on the medians of each exposure category and scaled to IQR increases in the original cumulative-days-of-use variables, for associations between depression and cumulative days of use of the fumigants ethylene dibromide and methyl bromide; the fungicide captan; and the organochlorine insecticide lindane in each case group (see Supplemental Material, Table S5). For none of these agents, however, were the categorical ORs monotonically increasing in each case group (see Supplemental Material, Table S5). We also observed positive trend ORs for several other pesticides in at least one case group and several pesticides had significantly different trend ORs at $\alpha = 0.1$ among case groups (see Supplemental Material, Table S5).

Augmenting models for ever-use of pesticide classes or individual pesticides by including additional variables (number of children, doctor visits in the past year, farm size, use of chemical-resistant gloves, cumulative lifetime days of use of any pesticide, or the pesticide that was most strongly correlated with the pesticide of interest) one at a time in models for all the different types of weights did not meaningfully change results, nor did including all variables in Table 1 and Supplemental Material, Table S1, in the models for the dropout weights (data not shown). Excluding applicators who reported physician-diagnosed pesticide poisoning did not change results (data not shown). We saw no consistent evidence of effect measure modification by state or by use of chemical-resistant gloves (data not shown). Finally, results were similar when we used standard regression methods (see Supplemental Material, Tables S6–S7).

Discussion

We found positive associations between use of some pesticides and depression among male private pesticide applicators in the AHS. Depression was positively associated in each case group with ever-use of two pesticide classes, fumigants and organochlorine insecticides, as well as with ever-use of seven individual pesticides: the fumigants aluminum phosphide and ethylene dibromide; the phenoxy herbicide 2,4,5-T; the organochlorine insecticide dieldrin; and the OPs diazinon, malathion, and parathion. Positive relationships between depression and cumulative days of use were evident, though nonmonotonic, in each case group for the fumigants ethylene dibromide and methyl bromide, the fungicide captan, and the organochlorine insecticide lindane.

Positive associations between depression and acute, high-intensity pesticide exposures, such as pesticide poisoning or high pesticide exposure events, were reported previously in a longitudinal study of 651 Colorado farmers and their spouses (Beseler and Stallones 2008) and cross-sectional studies of 208 Costa Rican banana plantation workers (Wesseling et al. 2010), and 17,585 male private pesticide applicators (Beseler et al. 2008) and 29,074 wives in the AHS (Beseler et al. 2006). In our study, depression was positively associated with physician-diagnosed pesticide poisoning and high pesticide exposure events among PRE-E and PRE-B cases, but not among POST cases.

Previous studies have observed positive associations between depression and exposure to any pesticides or to some pesticide classes, particularly OPs: a follow-up study in Brazil that compared 25 agricultural workers assessed after 3 months of OP exposure with themselves assessed again after 3 months of no OP exposure (Salvi et al. 2003); a 3-month follow-up study in Poland that compared 26 OP-exposed greenhouse workers with 25 unexposed canteen, kitchen, and administrative workers (Bazylewicz-Walczak et al. 1999); a 3-year follow-up study of 257 farm operators in Iowa that compared those exposed to pesticides with those who were not (Onwuameze et al. 2013); a cross-sectional study in England that compared 127 current and retired sheep dippers exposed to OPs with 78 unexposed current and retired police officers (Mackenzie Ross et al. 2010); and a cross-sectional study of 17,585 male private pesticide applicators in the AHS that separately compared those exposed to any pesticide or to seven pesticide classes (carbamates, fumigants, fungicides, herbicides, insecticides, organochlorine insecticides, OPs) with those who were not (Beseler et al. 2008). A study of 567 agricultural workers in France that evaluated exposure to any pesticide, three pesticide classes, or 13 herbicide families, using no exposure to the pesticide class/family in question as the reference, reported positive associations between depression and exposure to herbicides in general and dinitrophenol herbicides, but not exposure to any pesticide, fungicides, insecticides, or the other 12 herbicide families (Weisskopf et al. 2013). In contrast, a cross-sectional survey of 9,844 sheep dippers in England and Wales that used no exposure to any pesticides as the common reference found no association between depression and use of sheep dip (usually diazinon or other OPs), other insecticides, herbicides, fungicides, or wood preservatives (Solomon et al. 2007). In our study, depression was positively associated with cumulative days of use of any pesticide among PRE-E and PRE-B cases, ever-use of the pesticides classes fumigants and organochlorine insecticides in each case group, and ever-use of several other pesticide classes, including OPs, in at least one case group. Results appeared to be independent of pesticide poisoning, because we observed similar results when we excluded applicators who reported physician-diagnosed pesticide poisoning (data not shown).

Only one previous study evaluated the association between depression and a specific pesticide, finding a cross-sectional association between parathion exposure and CES-D scores indicative of clinical depression among 115 adults in Jackson County, Mississippi (Rehner et al. 2000). We found that ever-use or trend versions of cumulative lifetime days of use of several individual pesticides, including parathion, were positively associated with depression.

In general, we observed fewer positive associations between pesticide use and depression among POST cases than among PRE-E or PRE-B cases. Reverse causation—where depression increases exposure, perhaps through careless handling of pesticides—is unlikely to explain the differences in associations among case groups because use of chemical-resistant gloves was not inversely associated with depression after adjustment for age and state, and because including use of chemical-resistant gloves in models for the weights did not change results. Alternatively, differences among case group–specific associations might be attributable to exposure being evaluated closer to first reported diagnosis of depression for PRE-E and PRE-B cases than for POST cases, which could be particularly important for pesticides, such as organochlorine insecticides, with marked secular trends in use. Using information on past instead of ongoing pesticide use could have obscured associations with POST depression. Differences among case group–specific associations might be attributable to residual confounding from observed differences in personal characteristics or in cumulative days of use of any pesticide among case groups; for example, the average cumulative days of use of any pesticide reported by POST cases was 343 compared with 424 for PRE-E and 387 for PRE-B cases (Kruskal–Wallis $p = 0.02$).

Finally, although we asked about ever-diagnosis of depression at both enrollment and follow-up, some PRE-E depression cases were likely misclassified because they did not report a previous diagnosis at follow-up; in other words, they should have been classified as PRE-B cases. Possible reasons for this omission include recovering from depression before the follow-up interview (which was administered 12.1 years, on average, after enrollment) or, due to the sensitive nature of mental health conditions, being less inclined to confirm a previous diagnosis of depression because the follow-up interview was conducted via telephone, whereas depression information was collected at enrollment via self-administered paper questionnaires. We cannot, however, confirm either of these possibilities. Despite this possible misclassification, we analyzed PRE-E depression as a separate case group because the number of applicators in this group was large (n = 474) and associations with pesticide use differed from those observed with PRE-B depression.

We used three strategies to account for exposure to multiple pesticides. First, we grouped individual pesticides into 10 pesticide classes (4 functional, 6 chemical) because the pesticide that was most strongly correlated with the pesticide of interest was often in the same class. We also conducted sensitivity analyses in which we additionally weighted for cumulative days of use of any pesticide or for the pesticide that was most strongly correlated with the pesticide of interest. Although neither strategy meaningfully changed our results (data not shown), we cannot rule out the possibility that associations between depression and use of individual pesticides were confounded by use of other pesticides.

We used inverse probability weighting to adjust for potential confounding and for potential biases from missing covariate data, missing farmer questionnaires, or dropout. One limitation of inverse probability weighting is that residual confounding, missing data bias, and/or selection bias could still occur. In addition, c-statistics for the dropout models, while not used to select variables for inclusion in our models for the weights, ranged from 0.60 to 0.61, which suggests that dropout in the AHS is mostly random or that our models did not predict dropout well. The former seems more likely because Montgomery et al. (2010) found that applicators who reported physician-diagnosed depression at enrollment were equally likely to drop out of the AHS before the first follow-up interview in 1998–2003 as applicators who did not report depression (OR = 0.92; 95% CI: 0.82, 1.02 after adjustment for age, state, education, and smoking).

Our information on pesticide use was self-reported and could be misclassified. Using data from orchardists in Washington State reported during the year of use as the gold standard, Engel et al. (2001) found sensitivities for reporting ever-use of pesticides 25 years later were 1.00 for any pesticides, 0.87–1.00 for pesticides classes included in our study, and 0.80–0.94 for individual pesticides included in our study. A case–control study of cancer in Montreal, Canada, found the specificity of self-reported ever-exposure to pesticides or fertilizers was 0.95 when compared with expert assessment (Fritschi et al. 1996). In a reliability study of a subset of AHS applicators in Iowa who completed the enrollment questionnaire twice 1 year apart, percent exact agreement for ever-use of 10 individual pesticides ranged from 0.79 to 0.88 (Blair et al. 2002). Another study found that < 1–5% of AHS applicators overestimated duration of use of 19 individual pesticides relative to the years the pesticide active ingredients were first registered for use with the U.S. Environmental Protection Agency (Hoppin et al. 2002). The effect of depression on recall of past pesticide use is unknown. Cancer cases and controls, however, were found to report pesticide use with similar accuracy in a validation study in Kansas (Blair and Zahm 1993), and there is little evidence for differential recall in the self-reporting of occupational exposures among cases and controls of other diseases (Teschke et al. 2002).

We also relied on self-reports of ever physician-diagnosed depression. Using information from a validation study conducted in a cohort of university graduates in Spain, the calculated sensitivity and specificity of self-reported ever physician-diagnosed depression was 0.85 and 0.68, respectively, when the Structured Clinical Interview for the *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition*, was used as the gold standard (Sanchez-Villegas et al. 2008). In addition, associations we observed with pesticide poisoning and patient characteristics were similar to those reported in other studies, increasing confidence in the accuracy of our outcome. For example, depression was more common among applicators who were past smokers (Strine et al. 2008) or who had visited a medical doctor in the past year or had poorer health (Beseler and Stallones 2008). Therefore, the validity of self-reported ever physician-diagnosed depression in our study is likely good.

Our cohort is imperfect for longitudinal analyses of pesticide exposure and depression because we collected information on depression at only two points in time on average 12.1 years apart, and we assessed ever physician-diagnosed depression rather than current depression. Thus, we were unable to use longitudinal or life-course statistical methods.

Our study has several strengths, including its large size. Its prospective nature provided the opportunity to identify POST cases of depression as well as PRE-E and PRE-B cases. We had detailed information on applicators' exposures, including general pesticide exposure, use of pesticide classes, and use of individual pesticides. We could control for many potential confounders and demonstrated the robustness of our results to additional potential confounders not included in the main models (data not shown). Finally, we used inverse probability weighting to adjust for potential biases from missing covariate data, missing farmer questionnaires, or dropout. Overall, the effect of missing data and dropouts on our results appeared to be small because results were similar when we used standard regression methods (see Supplemental Material, Tables S6–S7).

Conclusions

Our study supports a positive association between depression and occupational pesticide use among applicators. Furthermore, it suggests several specific pesticides that deserve further investigation in animal studies and other human populations.

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BANGOR DAILY NEWS

Maine records first-ever human case of EEE

HANDOUT | REUTERS

A Culex quinquefasciatus mosquito on a human finger in this undated handout photograph from the Centers for Disease Control and Prevention (CDC).

By Jackie Farwell, BDN Staff
Posted Oct. 10, 2014, at 11:48 a.m.

A York County man has been confirmed as Maine's first-ever human case of Eastern Equine Encephalitis, a rare but potentially deadly disease carried by mosquitoes, according to the Maine Center for Disease Control and Prevention.

The man, whose name was withheld in accordance with privacy guidelines, first experienced mild symptoms in late July, Maine CDC Director Dr. Sheila Pinette said. In mid-August, he developed a fever, severe headache and confusion, and was hospitalized in York County for a week, then transferred to Boston, she said.

"The individual is home recuperating at this time, after a long hospitalization and rehab, and is doing fairly well with some mild [neurological] deficits," Pinette said.

The man is over age 60, she said.

Most individuals infected with the EEE virus experience no symptoms of illness, according to the U.S. CDC. But in 4 percent to 6 percent of diagnosed EEE cases, patients develop a severe form of the virus that causes neurological symptoms, such as brain swelling.

One out every three EEE patients with inflammation of the brain dies. Many survivors suffer memory, speech or cognition problems. Small children and older adults have a higher risk of developing neurological problems related to viral infections, Pinette said.

Blood samples collected from the York County man on Oct. 1 tested positive for the virus at a commercial lab, according to a Maine CDC health alert issued Friday. The state's testing lab subsequently confirmed the results on Oct. 9 and the sample was forwarded to the U.S. CDC for further confirmation.

Medical staff checked the patient for EEE in late August, but tests didn't detect the virus until October, Pinette said. Samples collected early in the course of the illness may come back negative.

While the EEE virus has been found in mosquitoes, birds and animals in Maine, the case marks the first confirmed time a human has contracted the disease in the state. Maine was among the last New England states to avoid a human case.

The illness reappeared in Maine after killing 15 horses in 2009. In 2012, a flock of 30 farm-raised pheasants in Lebanon died from EEE. Last year, the virus led to the deaths of two horses in Maine.

In August, Maine CDC announced a New Hampshire resident was hospitalized at Maine Medical Center in Portland with EEE. The patient contracted the viral illness in New Hampshire but needed the high level of care available at MMC, according to health officials. That individual later died, Pinette said.

A visitor to the state from Massachusetts died from the disease in 2008.

Among those infected with EEE, the illness begins with a sudden headache, high fever, chills and vomiting lasting one to two weeks. The illness may then worsen, causing disorientation, seizures or coma.

EEE has no cure. Treatment consists of supportive care, including mechanical ventilation, IV fluids, and medication to control seizures and reduce brain swelling.

This year, Maine CDC has detected the EEE virus in 22 mosquito testing pools in York County and an emu in Cumberland County that died from the illness. Seven mosquito pools collected on Sept. 30 tested positive for EEE, prompting the agency to extend the mosquito trapping season until Oct. 15, according to the alert.

Mosquitoes may remain active when temperatures are above 50 degrees.

A bond question due to go before voters in November would improve Maine's surveillance for EEE, according to officials with the University of Maine's Cooperative Extension.

The extension formerly assisted Maine CDC in monitoring mosquitoes for both EEE and West Nile virus, explained Jim Dill, a pest management specialist. But the extension was forced to stop in 2008 due to a lack of funding and U.S. CDC protocols governing safe handling of mosquitoes collected for testing, he said.

If Question 2, an $8 million bond initiative, passes in November, the cooperative extension plans to build a new facility in Orono to house labs for the monitoring and testing of insects and pests that afflict domestic and wild plants and animals in Maine. The planned facility includes a biosecure lab that would allow the extension to revive its mosquito traps in the northern part of the state and prepare the bugs for testing in Augusta, Dill said.

The state has tested about 400 mosquito traps this year from York, Cumberland, Oxford, Kennebec, Waldo and Aroostook counties, said Chuck Lubelczyk, a vector ecologist at the Maine Medical Center Research Institute, which partners with Maine CDC on testing. But some parts of the state — including Down East, the area between Rangeley and Jackman, and unmonitored parts of Aroostook — lack mosquito traps, he said.

“If you were to look at Maine in terms of a net, for surveillance, we would have more holes in the net than we actually have netting,” he said.

Surveillance of wildlife shows EEE occurs throughout the state, Lubelczyk said.

“The real question we don’t know is, why is it showing up so prevalently in wildlife and we have so few cases in those areas in humans?” he said.

Maine CDC issued Friday’s health alert in hopes of encouraging Mainers to consider EEE when spending time outdoors, including sportsmen heading into the woods to hunt, Pinette said.

“Prevention’s the key here … Our goal is not to alarm people, it’s just to make sure that they’re aware,” Pinette said.

Maine CDC recommends the following preventive measures to protect against mosquito-borne illnesses, such as EEE and West Nile virus:

— Avoid spending time outdoors at dawn and dusk when many species of mosquitoes are most active.

— Use an EPA-approved repellent when outdoors and always follow the instructions on the product’s label. Lemon eucalyptus oil is a natural alternative.

— For children under three, use netting on strollers.

— Wear protective clothing when outdoors, including hats, long-sleeved shirts, pants and socks.

— Use screens on windows and doors to keep mosquitoes out of the home, and patch any holes.

— Empty standing water where mosquitoes can breed, such as from flower pots, tire swings, buckets and barrels.

Maine confirms its first case of EEE in human

pressherald.com/2014/10/10/first-maine-resident-tests-positive-for-eee/

By Scott Dolan Staff Writer sdolan@pressherald.com | @scottddolan | 207-791-6304

The Maine Center for Disease Control and Prevention confirmed the state's first human case of neuroinvasive Eastern equine encephalitis Friday. The rare, mosquito-transmitted disease kills about one-third of the people who contract it, and can leave survivors with brain damage caused by swelling. It previously had been confirmed in other states, including two people this year in neighboring New Hampshire, but until Friday no humans had been reported to have contracted EEE in Maine since the state began testing in 1964.

"It's not a surprise," said Dr. Sheila Pinette, director of the Maine Center for Disease Control. "Eastern equine encephalitis has been in the state for a number of years. It was just a matter of time before the cases were going to occur."

Additional Images

The mosquito is a known carrier of Eastern equine encephalitis. The Maine Center for Disease Control has confirmed the state's first case of EEE in a York county adult. The Associated Press

Severe cases that involve inflammation of the brain are reported in five to 10 people nationwide annually, according to the federal Centers for Disease Control and Prevention. Symptoms, which appear four to 10 days after a person is bitten by an infected mosquito, can include the sudden onset of headache, high fever, chills and vomiting. Disorientation, seizures and coma can follow.

Maine's confirmed case was found in a York County adult over 60 who began feeling sick in late July and had to be hospitalized in August, first in Maine and then in Massachusetts as the disease worsened. The person, whom Pinette would not identify, is back in York County recuperating at home under family members' care, she said.

"My understanding is the neurological deficiencies are mild," Pinette said.

Initial tests for EEE were inconclusive when the York County patient was hospitalized on a respirator and under close watch. It can often take weeks for a person's body to create antibodies for EEE that can be detected in the bloodstream. Medical workers took another blood sample after the person had recovered, and that sample tested positive for EEE antibodies, first in a commercial laboratory on Oct. 1 and then again at Maine's Health and Environmental Testing Laboratory in Augusta on Thursday, Pinette said.

Since antibiotics are not effective against viruses, and no effective anti-viral drugs to combat EEE have been discovered, treatment for the disease usually relies on giving patients respiratory support and IV fluids while preventing other infections.

The Maine CDC announced in early September that the EEE virus had been detected in 22 mosquito pools in York County and in an emu in Cumberland County. The disease also had been detected in mammals and mosquitoes this year in New Hampshire and Massachusetts.

In previous years, the threat of EEE in other states had caused officials in some towns to restrict outdoor activities after dusk.

SPREAD AND PREVENTIVE MEASURES

The disease is spread when birds bitten by infected mosquitoes fly to another area and are bitten by other mosquitoes, who then spread the disease to other mammals, including humans. The disease is not transmitted person to person, and larger mammals are generally considered dead-end hosts because the concentration of the virus in their bloodstreams is usually insufficient to infect mosquitoes.

There is no vaccine or preventive drug for EEE and the best way to keep from getting it is to reduce exposure to mosquitoes by using repellent and wearing protective clothing when outdoors. A key tactic in fighting the disease is to eliminate the standing water around houses and yards where mosquitoes lay their eggs.

While the virus has only been detected in southern Maine, it is likely much more widespread in the state than that, according to Jim Dill, a pest management specialist with the University of Maine Cooperative Extension in Orono.

Dill said the state has only two biosecure laboratories that can regularly test mosquitoes and birds: the state lab in Augusta and a lab at Maine Medical Center in Portland.

Mosquito trapping and testing isn't being done in northern Maine, but testing on white-tailed deer confirms that the disease has spread.

"They pretty much found EEE in the deer everywhere in the state," Dill said.

A Democratic state representative from Old Town, Dill spoke in favor of a bond issue on the statewide ballot in the Nov. 4 election that would fund the creation of a biosecure laboratory to be run by the University of Maine Orono's Cooperative Extension Service.

Question 2 asks voters: "Do you favor an $8 million bond to support Maine agriculture, facilitate economic growth in natural resource based industries, and monitor human health threats related to ticks, mosquitoes, and bedbugs through the creation of an Animal and Plant Disease and Insect Control laboratory administered by the University of Maine Cooperative Extension?"

Dill said having that lab would allow the state to step up its mosquito testing in addition to the other services the lab would offer.

"If you think of people in Maine, we grew up with mosquitoes. It used to be, it's just a mosquito, squash it," Dill said. "It's not like that anymore."

TWO TYPES OF ILLNESS

EEE infection can cause two types of illness: systemic and encephalitic. The majority of cases are systemic infections, a milder disease that lasts about a week or two with fevers and chills but no nervous system involvement. Systemic infections often pass without hospitalization and usually go unreported, according to the U.S. CDC.

About 4 percent to 6 percent of those infected develop encephalitic EEE, which involves swelling of the brain and kills about one-third of those who contract it.

Another third suffer permanent brain damage and the remainder survive without serious complications, Pinette said.

“The spectrum is extremely broad and varies individually because each person is different,” she said.

The threat of infection drops greatly as the temperature drops with the approach of winter. Mosquitoes become inactive at about 45 degrees and below, Pinette said.

Maine officials will continue mosquito trapping until Wednesday, she said.

Repellency of Selected Chemicals Against the Bed Bug (Hemiptera: Cimicidae)

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ABSTRACT In recent years, the common bed bug, *Cimex lectularius* L. (Hemiptera: Cimicidae), became a major public health concern in urban communities. Bed bugs are notoriously difficult to control, and their bites are not tolerated by most people. The public has an urgent need for materials and methods to reduce bed bug introduction and bites during work, travel, or sleep. A repellent product will help achieve these goals by discouraging and preventing bed bugs from moving to a protected area. We evaluated the repellency of three commercially available insect repellent or control materials and five nonregistered materials with the goal of identifying safe and effective bed bug repellents. The two commercial repellent products that contained 7% picaridin or 0.5% permethrin had little repellency against bed bugs. *N,N*-diethyl-*m*-toluamide (DEET), the most commonly used insect repellent, provided a high level of repellency against bed bugs. When a host cue (carbon dioxide) was present, the minimum DEET concentration to repel ≥94% of the bed bugs for a 9-h period was 10%. The longevity of repellency of DEET was concentration dependent. At 25% concentration, DEET-treated fabric surface remained highly repellent to bed bugs for a 14-d period. However, DEET has a strong smell and dissolves certain plastic materials. Therefore, we evaluated several odorless, noncorrosive, and potentially effective repellents. Isolongifolenone and isolongifolanone, two natural products and recently reported insect repellents, exhibited strong repellent property against bed bugs but at significantly lower levels than DEET. Three novel potential repellent compounds discovered by Bedoukian Research Inc. (Danbury, CT) exhibited similar level of repellency and longevity as DEET for repelling bed bugs. These nonirritant and odorless compounds are promising candidates as alternatives to DEET for reducing the spread of bed bugs and bed bug bites.

KEY WORDS bed bug, repellent, DEET, natural product, essential oil

Since the late 1990s, bed bugs gradually reemerged as a common urban pest in the United States, Canada, Europe, Australia, and some Asian countries (Boase 2001, Hwang et al. 2005, Gangloff–Kaufmann et al. 2006, Doggett and Russell 2008, Kilpinen et al. 2008, How and Lee 2009, Hirao 2010, Wang and Wen 2011). Once introduced, eliminating bed bugs is both expensive and difficult. Pest control providers charge hundreds to thousands of dollars to control an infestation. The time to eliminate an infestation can take a few months or more, depending on infestation level, complexity of the environment, cooperation from the building occupants, and thoroughness of the treatment procedures. Given these challenges, preventing new bed bug introductions becomes an important issue to many people including residents, travelers, home care providers, social workers, pest control technicians, and others who may visit bed bug-infested environments. There is an interest for effective and safe repellent materials to help minimize the introduction and spread of bed bugs, and to reduce bed bug bites.

Insect repellents have long been used for preventing bites from blood-sucking arthropods (see review by Moore and Debboun 2006). DEET (*N,N*-diethyl-*m*-toluamide) is the most successful arthropod repellent in about six decades and has been the mostly widely used active ingredient in topical repellents to protect humans and livestock against variety of arthropods including mosquitoes (Robert et al. 1991, Fradin 1998, Qiu et al. 1998, Schofield et al. 2007, Syed and Leal 2008), biting midges (Harlan et al. 1983, Magnon et al. 1991, Young and Evans 1998), tabanids (Catts 1968), sand flies (Schreck et al. 1982, Coleman et al. 1993, Yaghoobi–Ershadi et al. 2006), black flies (Robert et al. 1992, Kalyanasundaram and Mathew 2006, Tawatsin et al. 2006), horse flies (Blume et al. 1971), chiggers (Lerdthusnee et al. 2003, Kitchen et al. 2009), ticks (Carroll et al. 2005, Zhang et al. 2009), and leeches (Kochhlar et al. 1974, Kumar et al. 1984, Tawatsin et al. 2006, Frances 2006a). The concentration

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of DEET used in a multitude of formulations around the world varies from 5 to 100% (Young and Evans 1998). Some side effects have been reported (Robbins and Cherniack 1986, Clem et al. 1993, Ross et al. 2004). DEET alternatives have always been sought and have been developed over the years as arthropod repellents. Useful repellents include permethrin, IR 3535 (3-[*N*-acetyl-*N*-Butyl] aminopropionic acid ethyl ester), *p*-menthane-3,8-diol, citronella, geraniol, picaridin, isolongifolenone, and isolongifolanone (Moore and Debboun 2006; Zhang et al. 2008, 2009).

Despite the increased importance of bed bugs in our society, there is only one report on effectiveness of repellents against bed bugs. Kumar et al. (1995) studied the repellency of DEET, diethyl phenyl-acetamide (DEPA), and demethylphthalate (DMP) against *Cimex hemipterus* (F.) by applying the chemical directly onto animal host skin. Both DEET and DEPA were repellent, with DEET being marginally more effective than DEPA.

Using an insect repellent can be a useful method to prevent bed bug bites, and possibly the introduction of bed bugs. Applying a repellent to shoes and pants may reduce the probability of getting bed bugs while a person is visiting an infested area. A repellent may also be applied to luggage, fabric materials, floors, or furniture to reduce the possibility of these objects becoming infested with bed bugs. An ideal bed bug repellent should prevent most of the bed bugs from crossing the treated area and last for at least a few hours or days. In addition, it should be odorless, nonirritating, and not an environmental pollutant. Many natural products and synthetic insecticides are claimed as bed bug repellents; however, there are no scientific data backing the claims. We evaluated the efficacy of several repellent products and chemicals with the aim of identifying effective and safe bed bug repellents. The evaluated materials included: 1) DEET—the most widely used insect repellent, 2) representative commercial products (active ingredients: permethrin and picaridin), 3) two recently reported natural repellent materials—isolongifolenone and isolongifolanone, and 4) three novel potential insect repellents developed by Bedoukian Research Inc. (Danbury, CT).

Materials and Methods

Bed Bugs. A laboratory (Ft. Dix) and three field strains (Essex, Indy, and Irvington) of bed bugs were maintained in plastic containers (47 mm in diameter by 47 mm in height) with folded filter paper as harborages. The laboratory strain had been originally collected from Ft. Dix, NJ, and maintained in glass jars (feeding on Dr. Harlan) since 1973. We obtained this strain from Dr. Harlan in 2009. The Essex, Indy, and Irvington strains were maintained in the laboratory for 6 mo, 2 yr, and 1 mo, respectively. Different experiments used same or different strains of bed bugs based on availability. The bed bugs were fed weekly with defibrinated rabbit blood (Hemostat Laboratories, Dixon, CA) using Hemotek membrane-feeding system (Discovery Workshops, Accrington, United Kingdom). The bed bugs were kept at 23–26°C, 24–48% relative humidity (RH), and a photoperiod of 12:12 (L:D) h environment. In all experiments, 7- to 21-d hungry bed bugs were used.

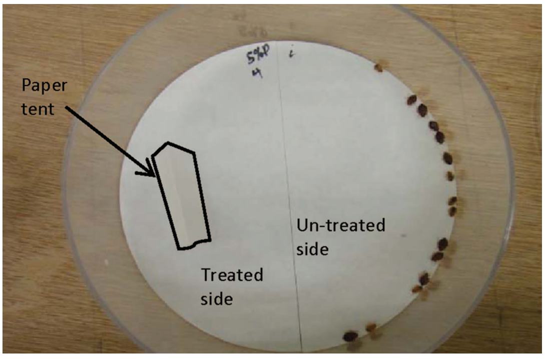

Fig. 1. Petri dish assay set up examining the repellency of candidate materials. Note all bed bugs were resting on the untreated side. (Online figure in color.)

Chemicals. DEET (97% purity) was purchased from Sigma–Aldrich Co. (St. Louis, MO) and diluted with 95% ethanol (Phamco Products Inc., Brookfield, CT) to desired concentrations. Cutter Advanced Insect Repellent (7% picaridin, United Industries Corporation, St. Louis, MO) and Rest Easy Bed Bug & Insect Control (0.5% permethrin, Eaton, Twinsburg, OH) were purchased from an internet-based vendor. Isolongifolenone was synthesized at Beltsville, MD (Wang and Zhang 2008). Isolongifolanone, chemical A (3-methyl-5-hexyl-2-cyclohexenone), B (propyl dihydrojasmonate), and C (γ-methyl tridecalactone) were provided by Bedoukian Research Inc. Chemical A has a mild peach-herbaceous odor. Chemicals B and C are almost odorless. These three compounds were potentially useful insect repellents based on laboratory assays by the manufacturer.

Petri Dish Assays. Plastic Petri dishes of 11.4 cm diameter by 3.8 cm height were used to quickly evaluate the comparative repellency of the following candidate chemicals: DEET, permethrin, picaridin, isolongifolenone, and isolongifolanone. Filter papers were cut into two equal halves; one half was treated with a repellent using a Potter spray tower at 2.47 mg/cm^2 or 0.61 gallon/1,000 feet^2 of ethanol solution. The other half was sprayed with 95% ethanol. A small piece of filter paper was also treated with the same repellent and folded to a tent shape with the treated side facing down. The paper tent was placed on the repellent treated side and the dishes were left uncovered throughout the assay (Fig. 1). In the control dish, one half of the filter paper and the harborage were treated with 95% ethanol. The other half of the filter paper was not treated. In the assay evaluating 2.5% DEET, 7% picaridin, and 0.5% permethrin, 10 Ft. Dix strain bed bugs (fourth-fifth instar nymphs or adult males of unknown age) were released in the center of each dish. The numbers of bed bugs on each side of the dish were recorded at 2 and 24 h after treat-

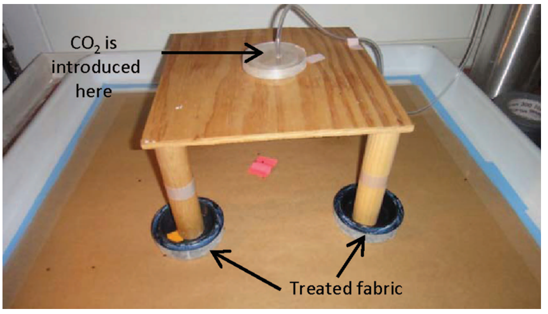

Fig. 2. Arena assay set up examining the repellency of candidate materials. (Online figure in color.)

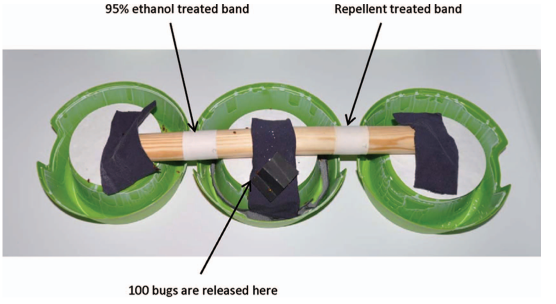

Fig. 3. Triple-bowl assay set up examining the repellency of 25% DEET. (Online figure in color.)

ment. In the assay evaluating repellency of 5% DEET, isolongifolenone, and isolongifolanone, nine males and six large nymphs of Essex strain bed bugs were released into each Petri dish. The location of bed bugs in each Petri dish was recorded at 3, 5, 9, and 24 h after treatment. Each treatment was replicated four times in both assays. The assays were initiated at ≈2–5 h into the dark cycle. The experiments were conducted in a room at 22–26°C and a photoperiod of 12:12 (L:D) h cycle.

Arena Assays. Plastic tray arenas (80 by 75 by 5 cm) with brown paper lining the bottom were used (Fig. 2) to evaluate the comparative repellency of selected chemicals when a host cue (carbon dioxide) is present. A layer of fluoropolymer resin (BioQuip products, Rancho Dominguez, CA) was applied to inner walls of the arenas to prevent the bugs from escaping. A piece of folded cardboard and folded fabric was placed at the center of the arena to provide harborages for bed bugs. A plastic ring (13.3 cm in diameter by 6.4 cm in height) was placed around the harborages to confine the bed bugs. Four arenas were placed in a nonventilated room at 24–25°C and a photoperiod of 12:12 (L:D) h cycle. They were served as four replicates. A 26.5 by 6.5-cm wooden stool was placed in each arena. Under the legs of each stool was a 10 cm in diameter by 2.2 cm in height black Climbup Insect interceptor (Susan McKnight Inc., Memphis, TN). An aliquot of 400 μl chemical solution was applied evenly to the fabric tape of each Climbup using a 200 μl pipette, yielding 5.3 mg/cm^2 or 1.1 gallon/1,000 feet^2 of chemical solution. The four Climbup interceptors in each arena were treated with four different chemicals with 95% ethanol being used as control.

In each test, bed bugs were released into the center of each arena and confined with a plastic ring. After 1 h and during the dark cycle, treated Climbup interceptors were placed under the stool legs and the rings confining the bed bugs were removed. Carbon dioxide (100% CO_2) was released from a gas cylinder (Airgas East Inc, Piscataway, NJ) to the top of each stool at 100 ml/min to stimulate bed bug activity. CO_2 was a strong stimulant to bed bugs (Wang et al. 2009). The number of bed bugs that fallen into each Climbup was recorded after 2–3 h or at other specified times. After each examination, all of the bugs scattered in the arena and Climbups were returned to the center of the arena, and confined for another 0.5 h; the room was ventilated to bring down the CO_2 concentration to the same level as the air within the room. The plastic rings confining the bed bugs were removed, and the bed bug numbers were recorded again following the same procedures to evaluate the repellency longevity of the chemicals.

The following comparisons were examined using the arena assay method: 1) comparative repellency of 25% DEET, isolongifolenone, and isolongifolanone at 4, 6, and 9 h after application (Essex strain, 100 male adults per arena); 2) comparative repellency of 5, 10, and 25% DEET at 3, 6, and 9 h after application (Essex strain, 60–70 male adults per arena); 3) repellency of 25% DEET at 1, 7, 14, 21, and 35 d (Indy strain, 50 male adults per arena); and 4) comparative repellency of 25% DEET, chemical A, B, and C (five-legged stools were used) at 0 d (Ft. Dix strain, counts were from 20-h test period) and 15 d (Bayonne strain, counts were from 6-h test period) after application. In the test examining the longevity of 25% DEET, two opposite legs of each stool were sitting on DEET-treated Climbups, whereas the other two legs were sitting on nontreated Climbups.

Triple-Bowl Assays. This experiment was designed to evaluate the efficacy of 25% DEET-treated bands for repelling bed bugs under conditions mimicking the natural environment. The experimental setup consisted of three inverted plastic dog bowl (600 ml in volume and 18 cm in diameter by 64 cm in height) (IKEA, Baltimore, MD), placed next to one another with a wooden rod serving as a bridge between the three bowls (Fig. 3). The inner surfaces of the dog bowls were coated with a layer of fluoropolymer resin to prevent trapped bed bugs from escaping. One piece of filter paper (10 cm in diameter) and a piece of black cloth were placed at both ends of the wooden rod to provide harborages for bed bugs. A piece of cloth was placed at the bottom of the center bowl to allow bed bugs trapped in the bottom to be able to climb back to the harborage located at the wooden rod, whereas bed bugs captured in either of the two side bowls could not return to the harborages associated with the wooden rod. Eight plastic containers, each with 100 Irvington strain bed bugs

(≈90% adult males and 10% fourth-fifth instar nymphs), were prepared 1 d before the test. The Irvington strain was selected for this experiment because the strain was only kept in the laboratory for 1 mo and the bugs were very responsive to host cues.

Two tests were conducted using triple-bowl devices. In the first test, 100 bed bugs were released into the center bowl at 2 h into the dark cycle. After 15 min of acclimation, two wooden rods were placed horizontally between the bowls to allow bed bugs to cross between the bowls. One wooden rod was wrapped with a 2.5-cm-wide repellent-treated fabric tape (Micropore surgical tape, 3M Health Care, Neuss, Germany). The other rod was wrapped with a 95% ethanol-treated fabric band as control. The chemicals were applied to the bands using the same method as described in the arena assay 1 h before the test. The experiment was conducted in a room at temperature between 27–29°C and lighted with a 25 watt transparent red light bulb. CO_2 (100% concentration) was released from three 5 lb CO_2 cylinders each at 100 ml/min to stimulate bed bug foraging movement. Bed bugs would naturally disperse both vertically or horizontally from the center bowl after being stimulated. The three CO_2 release points were ≈1.5 m above the test devices. Eight sets of devices were set up in the room. The number of bed bugs found in the two side bowls was counted after 2 h. Once counted, the bed bugs were returned to the center bowl and the wooden rods removed. The room was vented for 10 min using a fan.

A second test was initiated at 8 h after 25% DEET application using exactly the same materials and procedures as in the first test. This test was to determine whether the repellency decreased significantly compared with that observed at 1–3 h after application. The number of bed bugs found in the two side bowls was counted after 2 h. Seven replicates were included in this test.

Statistical Analysis. Repellency indices from Petri dish assays were calculated according to the formula: $\textit{Repellency index} = \frac{C-T}{C} \times 100$, where C = the mean numbers of bed bugs on the treated filter paper halves in all control dishes, and T = number of bed bugs on treated filter paper half in one test dish (Todd 2011). Repellency indices were compared using analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) test. The bed bug count data in arena assays comparing different chemicals were analyzed using Proc Glimmix based on mixed multinomial model with treatment period as the random effect. The arena assay and the triple bowl assay examining the changes in repellency of 25% DEET were analyzed by using Proc Genmod based on multinomial model with "replicate" as the random effect. All analyses were performed using SAS software (SAS Institute 2009).

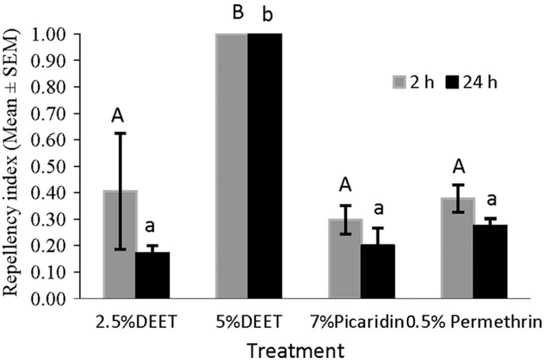

Fig. 4. Repellency of DEET and two commercial insect repellents against bed bugs in Petri dish assays. For each observation period, different letters above the bars of the same observation period indicate significant differences at $P = 0.05$.

Results

Petri Dish Assays. Bed bugs released into center of the dishes soon went under the paper tent harborage if the treatment was not repellent; or stayed along edge of the dish on the nontreated side if the treatment was repellent (Fig. 1). The 2.5% DEET, 7% picaridin, and 0.5% permethrin treatment exhibited low levels of repellency against bed bugs (Fig. 4). Only 5% DEET treatment achieved 100% repellency against bed bugs at 2 and 24 h after application. It was significantly more repellent than 2.5% DEET, 7% picaridin, and 0.5% permethrin (2 h: $F = 7.84$; df = 3, 12; $P = 0.0037$; 24 h: $F = 106.2$; df = 3, 12; $P < 0.0001$). Comparative tests of 5% DEET, isolongifolanone, and isolongifolenone revealed no significant differences in their repellency after 3 h ($F = 0.19$; df = 2, 9; $P = 0.83$). Isolongifolanone became significantly less repellent than DEET and isolongifolenone after 5 h ($F = \infty$; df = 2, 9; $P < 0.001$) and 9 h ($F = 62.8$; df = 2, 9; $P < 0.001$). There were no significant differences in their repellency at 24 h ($F = 3.48$; df = 2, 9; $P = 0.08$) after application (Fig. 5A).

Arena Assays. Comparative tests of 25% DEET, isolongifolenone, and isolongifolanone showed DEET was the most effective repellent (Fig. 5B). The ratio of the probability of bed bugs passed DEET-treated band vs. that passed isolongifolanone-treated band was 0.042 ($P < 0.0001$). The ratio of the probability of bed bugs passed DEET-treated band vs. that passed isolongifolenone-treated band was 0.028 ($P < 0.0001$). Individual comparisons at 6 and 9 h showed DEET was significantly more repellent than isolongifolenone and isolongifolanone ($P < 0.05$). However, the 25% DEET treatment did not completely prevent bed bugs from passing the treated surface. Among the bed bugs found in Climbups, 1% of them were found in 25% DEET-treated Climbups both at 6 and 9 h after treatment.

In concentration-repellency relationship assays, all tested concentrations exhibited significant repellency at 3 and 6 h after application (Fig. 6). At 9 h, the repellent effect of 5% DEET became insignificant ($P =$

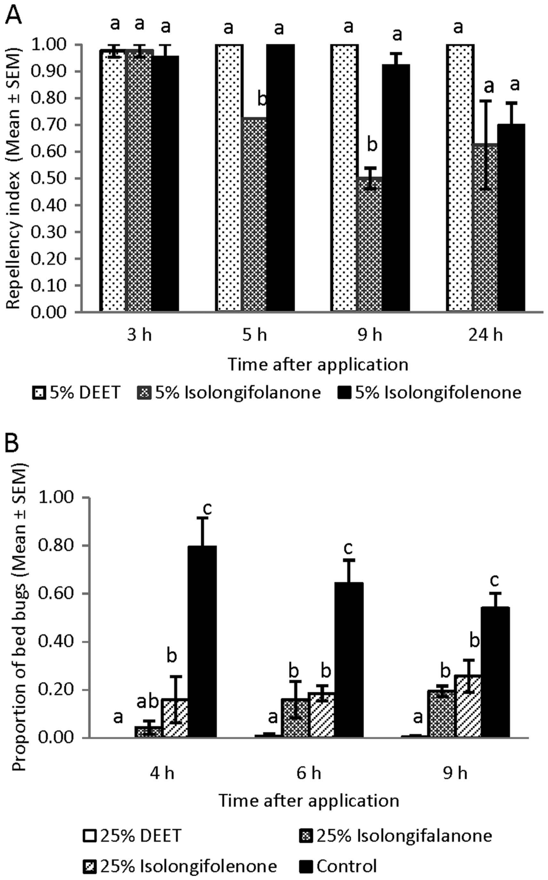

Fig. 5. Repellency of DEET and two recently patented insect repellent materials against bed bugs: (A) petri dish assay, (B) arena assay. Different letters above the bars of the same observation period indicate significant differences at $P = 0.05$.

0.17). Overall, the repellency of 5% DEET was significantly lower than 10% DEET ($P < 0.001$). There were no significant differences in repellency between 10%

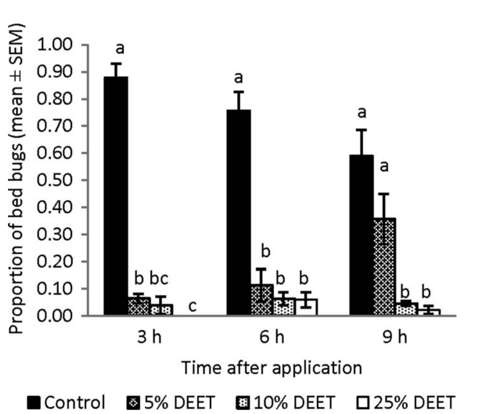

Fig. 6. Relationship between concentration and repellency of DEET against bed bugs in arena assays. Different letters above the bars of the same observation period indicate significant differences at $P = 0.05$.

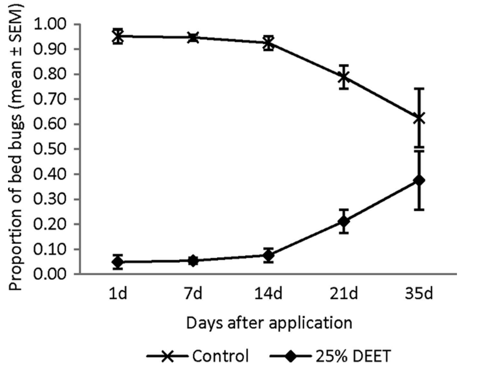

Fig. 7. Longevity of the repellency of DEET against bed bugs in arena assays.

DEET and 25% DEET ($P = 0.14$). Longevity tests of 25% DEET showed its repellency started to decrease significantly after 21 d ($P = 0.003$; Fig. 7). The percentage of bed bugs (mean ± SEM) found in 25% DEET treatment at 1, 7, 14, 21, and 35 d were 5 ± 2, 5 ± 1, 8 ± 3, 21 ± 5, and 38 ± 12%, respectively. At 35 d, the 25% DEET repellency was insignificant ($P = 0.19$). Chemical A, B, and C exhibited similar level of repellency as DEET at 0 d and 15 d ($P > 0.05$) (Fig. 8).

Triple-Bowl Assays. Bed bugs actively moved to the wooden rods once they were placed between the bowls. The vast majority of the bugs exhibited avoidance behavior when they reached the treated bands. No avoidance behavior was observed when bed bugs reached the control bands. In the first test (1–3 h after DEET application), the mean number of bed bugs appeared in the 25% DEET and the control side were 0.25 ± 0.3 and 41.4 ± 4.3, respectively. The DEET treatment side had an average 97 ± 1% less bed bugs compared with the control side. In the second test (8–10 h after DEET application), the mean number of bed bugs appeared in the 25% DEET and the control side were 1.3 ± 0.6 and 34.0 ± 3.5, respectively. The

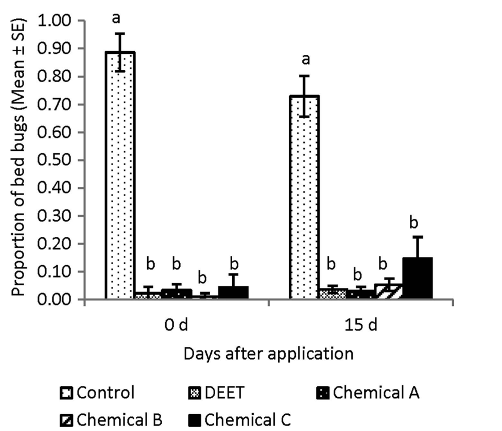

Fig. 8. Repellency of DEET and three potential insect repellents against bed bugs in arena assays. Different letters above the bars of the same observation period indicate significant differences at $P = 0.05$.

DEET treatment side had an average 94 ± 3% less bed bugs compared with the control side. There was no significant differences in the repellency measured at 3 and 10 h ($P = 0.14$).

Discussion

This is the first study addressing repellents for *Cimex lectularius* L. We found DEET and three compounds from Bedoukian Research Inc. are effective repellents against bed bugs. At 25% or higher concentration, DEET can prevent >94% bed bugs from crossing the treated area for at least 8 h under high pest pressure (i.e., hungry bed bugs and a strong host cue were present). The findings suggest that applying a repellent to luggage, shoes, or clothing could be an effective method to avoid bed bug infestations by home visitors, pest control technicians, travelers, and other personnel who need to visit or work in bed bug-infested environments.

We used three test methods to evaluate the repellent properties of candidate compounds. The Petri dish assay method provides a simple and fast method for screening large numbers of compounds. It is a more robust method than that introduced by Todd (2011), which does not contain harborages in the dishes. In that setup, bed bugs may randomly rest anywhere in the control dish, making it difficult to calculate the repellency index. In our Petri dish assays, 68 and 89% of the bed bugs stayed under the harborages in the control dishes at 2 and 24 h. Therefore, the repellency indices were more readily separated between treatments. Because there was not a host cue present in the Petri dish assays, the minimum effective concentration of chemical was much lower than that obtained from the arena assays. The arena assays mimic the field conditions where bed bugs from the floor need to climb a vertical substrate to reach the host. The drawback of this method is that the number of bed bugs falling into the Climbups was smaller than the number of bugs that reached the top of the interceptors because not all bugs reaching the top of the interceptors fell into the traps. It was not clear how many bed bugs crossed the treated fabric. The triple-bowl assays most closely mimic the natural conditions. While bed bugs can cross the wooden rods back and forth, once they fall to the bottom of the bowls, they cannot climb back. Most of the bugs (77% in the first test and 94% in the second test) in the side bowls were found at bottom of the bowls.

In several tests, we used same bed bugs repeatedly over time to determine the longevity of repellency. It is not clear whether bed bugs became less sensitive after previous exposure as shown in mosquitoes (Stanczyk et al. 2013). The repellency measured at a later time might be a combination of aging effect and changes in bed bug sensitivity. However, there is no evidence to believe prior exposure would affect the comparative repellency of the evaluated chemicals. From field application standpoint, the test design reflected the effectiveness of the repellents when bed bugs were continuously present. This repellency information is important to users who need to stay in an infested environment continuously for more than a few hours.

Permethrin is used as an effective repellent against a variety of biting insects by the U.S. military (McCain and Leach 2006). It effectively repels mosquitoes, sand flies, black flies, and ticks (Lindsay and McAndless 1978, Mercier et al. 2003). However, it exhibited low repellency against bed bugs at the commonly used rate. Similarly, Moore and Miller (2006) reported no significant repellency against bed bugs from several pyrethroid insecticides: λ-cyhalothrin, bifenthrin, and deltamethrin. Romero et al. (2009) found low level repellency from deltamethrin treatment. Thus, it is plausible that pyrethroids would not be good candidates as bed bug repellents. Picaridin has been shown to be as good as or better than DEET formulations for repelling mosquitoes (Frances 2006b). However, it only slightly repelled bed bugs. We tested 45% DEET using arena assays and found 100% repellency was never achieved in preliminary assays. These results suggest that bed bugs are more tolerant to insect repellents compared with some other blood-sucking arthropods.

Some essential oils were reported having repellent properties against blood-sucking insects. Among them, white cedar oil and peppermint oil were most repellent against mosquitoes (Barnard 1999). In a different study, we evaluated repellency of two essential oil-based bed bug control products using the arena assay method: EcoRaider (Reneotech Inc., North Bergen, NJ) and Bed Bug Patrol (Nature's Innovation Inc., Buford, GA). EcoRaider contains 1% cedar oil and Bed Bug Patrol contains 1% peppermint oil. Both these two products did not exhibit significant repellency against bed bugs (Singh and Wang, unpublished data). Based on these findings, it is unlikely that low concentration essential oils will be useful as bed bug repellents.

Isolongifolenone is a relatively new natural repellent material. Zhang et al. (2008, 2009) found it was equally or more repellent than DEET against two mosquitoes (*Aedes aegypti* (L.) and *Anopheles stephensi* Liston), blacklegged tick (*Ixodes scapularis* Say), and lone star tick (*Amblyomma americanum* (L.)) in laboratory assays. In the current study, this compound exhibited strong repellency against bed bugs but at significantly lower levels than DEET. Because it is natural product, it has high potential to be used as an alternative to DEET against bed bugs.

The comparable performance of the three chemicals from Bedoukian Research Inc. and the traditional DEET repellent is encouraging. These relatively new chemicals could be safer alternative repellents for preventing bed bug infestations than DEET. Increasing the band width from 2.5 to 7.5 cm did not improve the repellency in our preliminary studies. Thus, the 2.5-cm-wide bands were used in all repellent tests and we expect this width would be sufficient for personal protection under field conditions. These results imply that applying a narrow band of repellent may significantly reduce the probability of obtaining bed bugs while a human host is staying in a bed bug-infested room. This method could also be used to reduce the spread of bed bugs from an infested room to surrounding units in multiunit dwellings while waiting for treatment.

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