

Combined executive summary.

Project title: Improved breeding and variety evaluation methods to reduce acrylamide content and increase quality in processed potato products

Type of proposal: Coordinated Agricultural Project

Focus areas addressed:

Area 1. Research in plant breeding, genetics, and genomics to improve crop characteristics. Sub-area (a) product, taste, quality, and appearance. (40%)

Area 3. Efforts to improve production efficiency, productivity, and profitability over the long term (including specialty crop policy and marketing). (20%)

Area 5. Methods to prevent, detect, monitor, control, and respond to potential food safety hazards in the production and processing of specialty crops, including fresh produce. (40%)

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Critical stakeholder need addressed and the project's long-term goals

The US potato industry, with \$3.5 billion in raw product value, has identified acrylamide as the number one issue facing its long-term profitability and its number one research funding priority. The potato industry has a critical need for varieties that produce lower acrylamide in end products, while retaining or exceeding the agronomic and consumer acceptance traits found in current varieties. Improvements in potato breeding approaches and coordinated efforts for new variety adoption are required so that industry can respond rapidly to changing consumer preferences, improve raw tuber quality and reduce health concerns over acrylamide.

Summary of the outreach plan

New variety adoption has been a substantial challenge for the potato industry, in large part because processors and end users who write contracts that contain variety and tuber quality specifications have not been involved in the variety development process. The direct involvement of potato processors and end users in this SCRI project is unprecedented and includes industry funding of variety evaluation projects, use of their facilities for commercial scale production runs and participation in critical consumer attribute testing. This engagement changes the dynamic of the variety development process and greatly increases the likelihood that research findings and new potato varieties will be utilized by the processing industry. Leaders from all facets of the potato industry will play active roles in variety evaluation projects and will make key decisions with regard to variety assessment and advancement. Up to date findings will be communicated to the greater stakeholder community through regional workshops, presentations at highly attended winter commodity schools, national potato and horticultural conferences, articles in trade journals and a searchable database of variety performance characteristics accessible from the Internet.

Summary of potential economic, social, and environmental benefits

An objective of this research is the quantification of potential economic and societal benefits and risks associated with the adoption of new potato varieties that produce low acrylamide end products. Environmental benefits include protecting ground water quality and availability, and decreasing fuel used for shipping raw tubers. New potato varieties with improved nitrogen and water use efficiency compared to varieties such as Russet Burbank could improve the environmental impacts of potato production. Furthermore, improving raw product quality attributes will increase recovery and the production, transportation and processing efficiency of potato, reducing carbon footprint, greenhouse gas emissions and energy consumption of potato production and processing as a whole.

Summary of stakeholder engagement throughout the project

In response to the critical need to improve varieties, stakeholders organized and provided funding for potato variety trials for chip production in 2009 and for French fry production in 2010. Potato growers and processors also met with US Food and Drug Administration during the summer of 2010 to discuss accomplishments to date in mitigating acrylamide in processed potato products. United States Potato Board and National Potato Council leadership contacted project P.D. in September 2010 to request submission of a coordinated national proposal with focus on mitigating acrylamide. Prioritized goals for the project were developed with input from nearly 40 growers and processing company leaders from every production region of the country. Project development was facilitated by four conference calls hosted by the National Potato Council from November 2010 through January 2011, six conference calls in 2011 and 2012, meetings of the project P.D., Co-P.D.s and members of the advisory committee at the National Potato Council Expo in January 2011 at Las Vegas, NV and in January 2012 in Orlando FL, and multiple emails soliciting advisory committee feedback. The project extends the reach of industry-funded variety trial initiatives through cooperative efforts with the leadership of those activities. Project implementation, strategic decisions and assessments of progress are done in partnership with an advisory committee made up of influential potato growers, heads of state and regional potato organizations, and representatives of food-processing companies and end-user organizations.

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1. Introduction

1.1. Long-term goals and critical needs

Potato is the most consumed vegetable in the US, with production valued at \$4.0 billion. The long-term goal of this project is to facilitate the rapid, efficient development and adoption of new potato varieties that have exceptional agronomic, processing and consumer acceptance traits. An immediate critical need is to proactively reduce the acrylamide content of processed potato products in order to mitigate health concerns that have arisen with regard to acrylamide in food, and to remove the economic uncertainty associated with potential regulatory actions focused on acrylamide. Other critical needs addressed are those for rapid incorporation of desirable traits into potato breeding clones and for rapid industry response to emerging challenges and changing consumer preferences. ***Specific outreach and research objectives include*** (1) expanding variety evaluation efforts with a focus on acrylamide reduction and new variety adoption; (2) defining criteria for tuber composition that will allow industry to reduce acrylamide to as low as reasonably achievable (ALARA) levels; (3) identifying single nucleotide polymorphism (SNP) markers linked to high value traits to more efficiently develop improved varieties in the long-term, (4) evaluating the costs, benefits, and risks of introducing new varieties, and quantifying consumer preferences and relative valuations of new varieties with novel attributes including low acrylamide; and (5) evaluating the best new varieties at multiple locations at a scale that allows for robust, system-wide assessment and creation of guidelines for best management practices.

1.2 Stakeholder engagement

Engagement in defining goals for the project. The proposed research and extension activities, the criteria to evaluate progress, and the process for conducting cooperative activities have been developed with industry. Leaders at the US Potato Board (USPB) and National Potato Council (NPC) identified the need for new varieties with low acrylamide forming potential as the number one concern of the US potato industry and encouraged lead researchers to consider a strategy for acrylamide reduction focused on variety improvement. Variety evaluation efforts funded by industry that build on US public potato breeding programs served as models and logical starting points for this proposal. The proposed project expands the scope of these efforts through coordinated variety evaluations at a national level, and extends these activities by developing the necessary infrastructure for molecular breeding in potato, identifying lines for targeted breeding of low acrylamide varieties, and economic analysis of adopting new varieties.

Project implementation and the role of the advisory committee. The NPC and USPB leadership asked individuals representing the varied facets of industry to serve on an advisory committee for this project. Those industry members quickly agreed, and this highlights the importance of the proposed objectives to industry. The advisory committee for this proposal is unprecedented in its breadth. It includes influential growers, heads of state grower organizations, and representatives of four frozen French fry processing companies, four chip processing companies, and end users including McDonalds and Frito-Lay, the largest users of US potatoes for frozen processing and chip processing, respectively. This committee was assembled in Nov 2010 and elected its leadership on Dec 3, 2010. Sub-committees with focus on specific research and extension areas were defined in May 2012. Project plans were during face-to-face discussions with research leaders at the NPC-sponsored, industry wide Potato Expo on January 5, 2011 and January 4, 2012. The advisory committee will play critical, ongoing roles in the implementation, assessment and advising of project activities.

1.3 Body of knowledge

Acrylamide in food is a safety concern. Acrylamide is a neurotoxin, a carcinogen in rodents, and a suspected carcinogen in humans. In 2002, acrylamide was discovered in carbohydrate rich foods processed at high temperatures including potato chips, French fries and other processed products that together account for over half of US potato consumption. Acrylamide forms in a chemical reaction between asparagine (asn) and the reducing sugars glucose and fructose. The amount of acrylamide in potato end products depends on genotype, field management practices, field environment and processing methods. Production lines for French fries, potato chips and other products have been modified to lower acrylamide to the extent possible without degrading consumer acceptance attributes such as taste and texture. These efforts have been insufficient to consistently meet acrylamide targets, such as those established by the State of California.

There is uncertainty about future regulation of acrylamide in food. Concerns about acrylamide led to a court settlement with the state of California in 2008 in which processing companies and restaurant chains paid \$2M in fines and agreed to reduce acrylamide in processed products. The Food and Drug Administration intends to issue guidance for industry on reduction of acrylamide levels in food products. Industry members have indicated to the FDA that further acrylamide reduction depends on adopting new potato varieties that have consistently low reducing sugars and low asn in tubers. To address these emerging national priorities, targeted breeding and selection efforts need to be focused on identifying varieties that produce processed potato products with less acrylamide in the near term and ALARA amounts in the longer term.

Many tuber quality defects are caused by elevated reducing sugars. Managing reducing sugar accumulation in tubers is critical for end product quality. Reducing sugars react with amino acids at high temperatures in a non-enzymatic Maillard reaction to produce dark-colored, bitter-tasting products that are undesirable to consumers and industry. Acrylamide is a byproduct of these reactions and high acrylamide content is associated with dark fry or chip color. Many persistent and costly quality defects of potato result from elevated reducing sugars including cold-induced sweetening, sugar-end and stem-end chip defects and senescence sweetening. Since tubers with low acrylamide forming potential will have low reducing sugar contents, substantial relief from these quality defects is a synergistic benefit of the proposed activities.

The potato industry has a critical need for varieties that consistently produce high quality products. To be readily adopted by the processing industry, new varieties must increase the recovery of premium quality, high value products. The economic losses and waste resulting from having 10-20% or more of the harvested crop unsuitable for premium product production are no longer acceptable. New processing varieties must minimize health and regulatory concerns related to acrylamide and should enable industry to more rapidly adopt sustainable practices. To be acceptable to growers, new potato varieties must consistently produce a high quality crop. Crops that don't meet quality specifications under contracts typically have to be sold at much lower prices on alternative markets. Potato farms that fail to meet quality standards risk bankruptcy and a decrease in the socio-economic health of rural communities. Potato growers and processors have committed millions of stakeholder dollars to variety development because of the overriding need for germplasm tailored to fit twenty-first century demands including the need for low acrylamide in finished products.

Traditional potato breeding and variety evaluation methods have been slow and inefficient.

Potato breeding is conducted at 11 public programs in the US. Frito-Lay develops proprietary chipping potatoes in the only private US program. Potato breeding programs evaluate thousands of clones for agronomic, processing, and pest/disease resistance traits over many years. The process from first year of selection to the release of a new variety requires 10-15 years. At present, only a few molecular markers linked to disease resistance traits are available that could speed up the process of selecting parents and evaluating progeny. The 8,303 validated SNP developed in the SolCAP project will dramatically accelerate the pace of germplasm evaluation if SNP markers are linked to high-value traits. Large-scale commercial evaluations essential for acceptance of a variety by industry are only conducted during the last stage of variety development because of severe cost constraints related to seed production. Weaknesses in a variety may not be identified until after it is released, and this slows variety adoption by industry. The predominant frozen processing variety and most widely planted cultivar is Russet Burbank, which dates to 1908 and accounts for over 40% of the 423,000 hectares of US potatoes. For the potato industry to decrease acrylamide in potato products, respond to the marketplace and remain sustainable, potato breeders need the capacity to produce new varieties more rapidly and efficiently using high throughput selection methods and variety trials that incorporate increased replication across locations to quickly assess phenotypic variability and trait stability. Variety development efforts must take a system-wide view that seeks to exceed expectations for agronomic performance, processing quality, nutritional quality and consumer acceptance.

Potato has never been bred for low asparagine. Asn is the most abundant free amino acid in tubers and it is synthesized by asparagine synthetase. Two genes for this enzyme contribute to activity in tubers. Genetic variation for tuber asn exists in cultivated potato and wild species, but asn in many cultivated varieties is unacceptably high. Greater amounts of nitrogen fertilizer are associated with higher amounts of asn at harvest. To further reduce acrylamide in end products requires identifying breeding lines and breeding parents with low tuber asn, determining heritability of tuber asn, and developing nutrient management guidelines for new varieties to minimize asn in tubers.

Tuber reducing sugar contents reflect genotype and environment during growth and storage.

Four enzymes are primarily responsible for reducing sugar accumulation in stored potato tubers. They are UDP-glucose pyrophosphorylase (UGPase), sucrose-6-phosphate synthase, sucrose-6-phosphate phosphatase and vacuolar acid invertase (AcInv). Reducing sugar contents are lowest when A-II isoforms of UGPase are present and AcInv activity is low. Sucrose synthase influences sucrose conversion to starch during tuber bulking but has low activity post-harvest. UGPase and AcInv activity increase in response to environmental stress including storage temperatures less than 10°C. These two enzymes are the primary control points for cold-induced sweetening (CIS), sugar-end defects and stem-end chip defects. Climate, cultivar, and management practices including the extent and timing of nitrogen fertilizer application and crop maturity at harvest interact to influence tuber reducing sugar amounts post-harvest.

1.4. Significant activities related to the proposed project

This project builds on three highly significant efforts, the USDA AFRI SolCAP project; the National Fry Processor Trial (NFPT); and the National Chip Processor Trial (NCPT). Industry leaders from these efforts are members of our advisory committee to maximize coordination and prevent redundant activities. Most Co-PDs are participating in one or more of these efforts.

Increasing the reach of these existing efforts makes efficient use of scarce resources and will enable each to have a greater impact on the US potato industry.

SolCAP. The SolCAP project has developed SNP markers and genotyping platforms for potato. These have been used to genotype 325 lines representing current and historical cultivars and 199 individuals in a mapping population from a cross of Premier Russet, a fry processing variety, and Rio Grande Russet, a fresh market variety. This project is designed so that SNP marker associations and linkages to phenotypic data can be developed if phenotypic data become available. Co-PDs are carrying out SNP-genotyping of additional mapping populations.

National Fry Processor Trial. A consortium of growers, frozen product processors and state organizations first agreed to this effort in May 2010. The goal is to characterize advanced breeding lines with respect to acrylamide forming potential and acrylamide precursors. Feedback on the draft plan occurred on December 14, 2010 at a meeting of industry representatives, potato breeders and researchers in Chicago. The program evaluates 75 to 100 advanced lines at three (ID, WA, ND) locations. Tuber asn, sugar content, and acrylamide in fries are quantified to establish the range of these traits in cultivars and advanced lines.

National Chip Processor Trial. Growers identified a critical need for a chip potato cultivar that maintains quality in storage through May. A second focus on heat-tolerant varieties for use in Southern states was added subsequently. Project planning began in 2008 and trials with 220 early generation lines and select varieties began in 2010 in NY, ND, MI, WI, NC, FL, TX, and CA. Evaluation criteria include tuber yield, shape, specific gravity, disease resistance, and chip color.

1.5 Summary of progress to date

Objective 1. The NFPT evaluated 75 advanced fry processing clones in non-replicated trials in ID, WA and ND. Sugar and asn content of tubers and acrylamide content in finished fries were quantified shortly after harvest and after tubers were stored for three months. Data were disseminated to industry and researchers and discussed at the 2011 NCCC84 meeting of potato breeders in Chicago on December 12-13, 2011. Select clones were processed into fries at the JR Simplot pilot plant in Caldwell, ID. Fries were evaluated using quick service restaurant (QSR) specifications. A summary report describing the data was prepared by Simplot technical staff. The NFPT was expanded in 2012-2013 with additional trial sites in WI and ME and 88 clones.

The NCPT evaluated approximately 200 early generation clones in non-replicated trials at 10 locations in 2011-2012. Additional sites in OR and TX were added for the 2012-2013 trial. Clones were evaluated for tuber appearance, sugar content, chip color, and specific gravity. Data were discussed at the 2011 NCCC84 meeting and promising clones were advanced to the USBP/SFA trial for in depth evaluation. That trial had 15 clones at 11 locations in 2011-2012 and has been planted at 13 locations in 2012-2013 with 16 clones. Clones from the USBP/SFA trial with greatest potential for meeting goals established by industry were processed into chips at commercial plants operated by Barrel O'Fun Snacks, Better Made Snack Food, Inventure Foods, Kettle, Shearers Foods, and Utz Quality Foods and used for taste tests and acrylamide determinations. Taste test data are highly encouraging in that panelists were unable to taste differences between established varieties and new clones in most cases.

Objective 2. Double silencing, low-invertase, low-asparagine synthetase lines of Atlantic and Russet Burbank have been generated using two approaches. First, invertase-silencing lines have

been transformed with an asparagine synthetase-silencing construct. Second, additional lines have been generated using a novel double silencing construct. These lines are expected to have a range of tuber reducing sugars and asparagine contents and will have high utility for creating predictive models of acrylamide content of products based on precursor content in tubers. Low-invertase lines of Atlantic and MegaChip were evaluated out of storage in 2011-2012 for tuber processing quality and for variation in expression of genes for key enzymes of carbohydrate metabolism in potato tubers. Preliminary analysis of these data show that silencing the vacuolar acid invertase gene had little to no effect on the expression of other genes in the starch-to-sugar pathway in potatoes. A second year field trial was planted in May 2012.

Objective 3. Forty three clones and their parents from the russet mapping population developed and genotyped under the AFRI-funded SolCAP project were planted in replicated plots in Idaho and Michigan and will be used to confirm SNP marker associations for key fry processing traits. Tubers from the SNP-genotyped, chip mapping population (Tundra x Kalkaska) were placed in 45°F storage until February 2012 for chip processing and sample collection. Tuber tissue was freeze-dried for sugar and asn analysis from two replications. The chips are saved and may be used for acrylamide analysis. QTL analysis is planned when the sugar, asn, and chip color data are collected and quality checked. In May 2012, the chip mapping population was replanted for a second year of the study in a randomized complete block design with four replications. Tubers will be harvested in the fall, stored and then chip-processed to evaluate acrylamide-forming potential and agronomic traits.

Tuber tissue from the SolCAP diversity panel, which includes current and historical cultivars, advanced clones and breeding lines, was used to determine the range of tuber asparagine content in US germplasm. Mean asparagine content was 14 mg g⁻¹ DW, and a larger than expected range of values was observed. Minimum tuber asparagine observed was 2.7 mg g⁻¹ DW and maximum was 28.8 mg g⁻¹ DW. Recent introductions spanned the complete range, supporting the hypothesis that breeding efforts have not selected for high or low tuber asparagine content.

Objective 4. Data availability from public sources regarding potato management was assessed, including recent USDA NASS ARMS survey for fall potatoes in 7 major potato states and input price data from USDA NASS. Existing data on tuber growth and size have been compiled and analyzed, which will be part of the economic analysis for the agronomic evaluations. Methods of analysis are being developing for using data from standard variety trials to predict the size distribution of tubers and how field management can affect tuber size distribution. This will be part of the economic analysis for the agronomic evaluations conducted in Objective 5.

Objective 5. Trials are underway in ME and WA to assess how physiological maturity of tubers affects processing quality and acrylamide-forming potential. Physiological maturity is varied in these trials by supplying nitrogen (N) and potassium (K) at different rates. In-season sampling is planned to develop information on tuber maturation in the N experiments. Data to be collected at harvest and out of storage include yield, tuber size profile and specific gravity, internal and external defects, fry color, and biochemical indicators of tuber quality and acrylamide forming potential. Results will reveal how N nutrition and tuber maturity affect levels of acrylamide precursors at harvest and during storage. These trials are establishing protocols for advanced agronomic trials, which will focus on clones with low acrylamide-forming potential.

Project Narrative

2.1. Rationale and Significance

Acrylamide in processed potato products is the highest priority concern of the US potato industry. The activities proposed here address this concern using a system-wide approach that employs research, extension and outreach activities to deal with critical needs related to the production, processing and consumer acceptance of potatoes. Relationships of the proposed objectives to specific areas of effort are indicated in Figure 1.

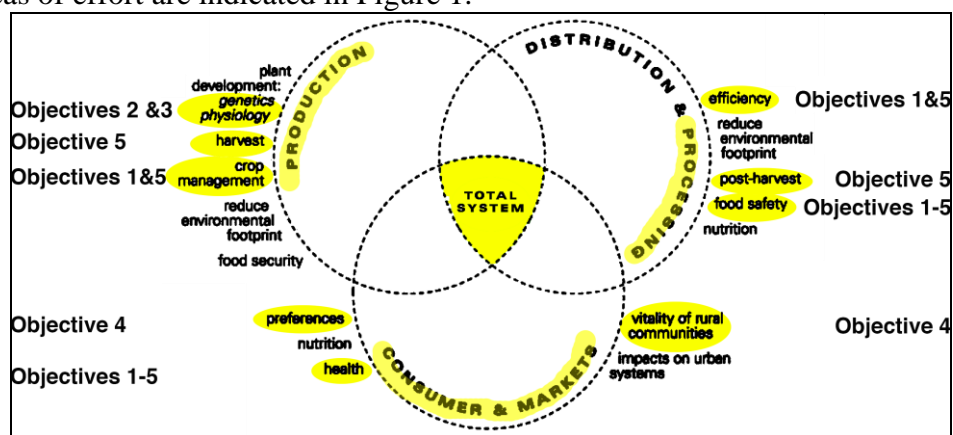


Figure 1. Proposed SCRI objectives are aligned with focused science and application study areas that support production, processing, consumers and marketing areas of the US potato industry.

Acrylamide was discovered in foods in 2002 and concerns about potential health risks were raised immediately. The effect on human health of long-term exposure to low amounts of acrylamide, such as those in food, is a subject of ongoing research worldwide. Regulations on acrylamide in food are being discussed, but at this point it is unclear how they will be structured or what limits, if any, will be established. Because the FDA and European Food Safety Authority consider acrylamide to be a food safety concern, the potato industry as a whole has come together to support a concerted effort to reduce acrylamide in processed potato products in the near term and to bring acrylamide down to ALARA levels in the longer term. This focused, cooperative activity is unprecedented in its scale and inclusiveness within the potato industry. This proposal is an integral part of that effort and the activities proposed here leverage and augment industry- and government-funded initiatives to achieve quantifiable results in the near term and lay the foundation for greater improvements over the long term. ***All aspects of this proposal address Focus area 5 in that the proposed activities seek to control, and respond to potential food safety hazards in the production and processing of the potato crop.***

The research and extension activities proposed provide critical support to the potato industry in the areas of potato breeding and variety evaluation. Key to the activities proposed is the recognition that the potato industry is highly integrated and that new varieties impact all aspects of the industry, including seed producers, commercial growers, processors, and consumers. Successfully introducing improved varieties that meet the expectations of the industry requires a systems approach to variety assessment that involves industry members at all levels in defining criteria for new varieties and evaluating promising lines. ***The extension and outreach activities proposed in Objectives 1 and 5 are directly applicable to Focus Area 3 in that they seek to***

identify, evaluate and characterize new potato varieties through a focused, coordinated, in-depth, national evaluation process that fully engages the industry.

New varieties with improved quality and low acrylamide forming potential will benefit the potato industry by reducing food safety concerns and minimizing waste caused by inconsistent raw product quality. Adopting new varieties requires investment and entails risk. The economic value and risk of new varieties to growers and processors will be assessed through research in these areas. The value of new varieties to consumers will be quantified through research using consumer auction methods. ***The economic research in Objective 4 promotes the goals of Focus Area 3, especially in the areas of profitability over the long term and marketing, by providing industry with analyses that will guide decisions related to variety adoption.***

A systematic evaluation of advanced breeding lines and recent cultivars will identify lines with high potential for reducing acrylamide in the near term. Achieving the longer-term goal of reducing acrylamide to ALARA levels will require directed breeding and selection. Developing molecular markers linked to high value traits in tetraploid potato is one way to increase the velocity of potato breeding programs. The genetics underlying acrylamide formation are uncertain, especially since potato has never been bred for low acrylamide or the acrylamide precursor asn. Objectives 2 and 3 include research on defining quantitative plant breeding targets for lines with low acrylamide forming potential, determining the heritability of asn in potato, and producing a set of SNP markers linked to high-value traits for use with elite potato germplasm. ***Objectives 2 and 3 are aligned with Focus Area 1 as they incorporate fundamental and applied research in plant breeding to improve crop traits, especially taste, quality and appearance.***

2.2 Approach

This project seeks to accelerate the adoption of improved potato varieties by the commercial sector with the goals of reducing the acrylamide content of processed potato products in the near term and decreasing acrylamide to ALARA levels in the future. Our approach leverages existing activities within the potato industry and research community to substantially increase their benefit. Objectives 1 and 5 have been developed in consultation with industry leaders who recognized that the impact of privately initiated variety trials could be increased substantially by conducting more complete evaluations that incorporate a system-wide approach to variety evaluation. The partnership proposed would facilitate additional assessments for all lines including those for end-product quality, consumer acceptance, acrylamide precursors in raw product, and acrylamide content in finished product (Fig 2 upper). To facilitate the commercial scale trials required to address risks associated with acceptance of new varieties, breeding programs will be given assistance in virus removal and seed multiplication. Many Co-PDs including Knowles, Pavek, Olsen, Thornton, Charlton, Bussan, Porter, Douches, Novy and Yenko will participate in these national field trials and extension research efforts. This approach reduces redundancy, increases efficiency, and by conducting replicated, large-scale evaluations, across multiple climates greatly increases the likelihood that new varieties with low acrylamide forming potential will be adopted by industry.

	Chip varieties	Fry varieties	
Tier 1	NCPT. 200+ lines at 8 locations. SCRI adds one location. Targets processing quality from field and after storage.		
Tier 2	NCPT. ~70 lines at 8 locations. SCRI adds one location. Targets processing quality from field and after storage.	NFPT. ~70 lines at 3 locations. SCRI adds 2 locations. Targets asn, acrylamide and processing quality from field and after storage.	Produce virus free tissue culture plants
Tier 3	National yield and quality assessment trials. 15 to 20 lines for chip and processing potatoes evaluated in larger scale replicated trials. Gives growers and processors a closer look at agronomic performance and quality attributes.		Grow from clean seed 250-300 kg seed tubers
Tier 4	Commercial scale production, storage and processing trials. 2 to 6 chip and 2 fry lines evaluated at 2 locations. Generate disease free seed to plant 2.5 ha of each line. Grow 150,000 kg of potatoes for each line to conduct commercial storage trials and processing in chip or fry plants.		Grow from clean seed 10-15 Mg seed tubers

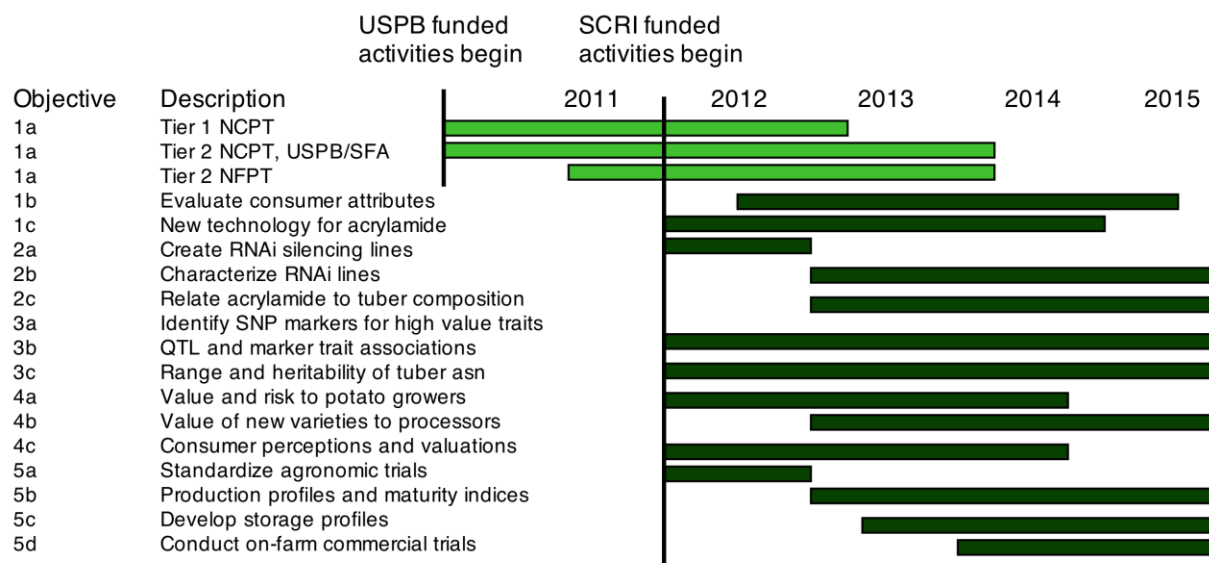


Fig 2. Upper: Commercialization process for new potato varieties that meet acrylamide, agronomic, processing performance and consumer acceptance standards. Tiers 1 and 2 are part of Objective 1. Tiers 3 and 4 are part of Objective 5. Activities with light green borders are primarily industry funded. Activities with dark green borders are USDA SCRI and industry funded. Lower: Gantt chart indicating project timeline.

Of the three primary research objectives, Objective 2 builds on basic research and existing research materials of Jiang and Bethke and uses molecular techniques to define quantitative goals for reducing sugars and asn in potato tubers that achieve ALARA levels of acrylamide. Objective 3 extends the SNP marker development, genotyping and phenotyping efforts led by Novy and Douches in the SolCAP project to add phenotypic data related to acrylamide and asn, undertake QTL and association mapping studies, and develop a suite of SNP markers linked to key agronomic traits that can be used by potato breeders. In Objective 4, Mitchell will quantify the economic trade offs to growers associated with introducing, or failing to introduce, new potato varieties that have improved tuber quality and low acrylamide in processed products. Guenther will extend this analysis and quantify the value to processors. Although we are not proposing to introduce GM potatoes, the market-based economic research by Huffman will define consumer attitudes toward low acrylamide potatoes produced through conventional breeding or GM techniques.

2.3. Objective 1: Implement a coordinated, nationwide evaluation of varieties with potential to reduce acrylamide in processed products.

	Goal	Acceptable
Agronomic traits		
Yield	≥regional average	
6 oz or more	70%	68-74%
10 oz or more	32%	28-40%
External defects	<standard variety	
Quality traits		
Specific gravity	1.084	1.082-1.088
Tuber asparagine	≤100 mg 100 g ⁻¹ FW	TBD
Reducing sugars	<2.5 mg g ⁻¹ DW	TBD
Internal defects	0%	0-2%
Bruise	80% Bruise free	70-100%
Length:width ratio	1.75:1	1.6:1-1.9:1
Processing traits		
Acrylamide	100 ppb	<200 ppb
Texture	Equals standard	
Flavor	Equals standard	
Color	Equals standard	

Table 1. Goals for agronomic, tuber quality and processing traits.

Critical need. Potato breeding programs across the US have many promising lines with potential for commercialization. Rapidly identifying new varieties with low acrylamide forming potential and exceptional agronomic traits requires a comprehensive evaluation program that replicates trials over locations to rapidly assess genotype by environment interactions. The goal of this objective is to identify 10 to 15 superior lines for chips and fries that will be entered into commercial development activities in Objective 5.

2.3.1: Sub-objective 1a. Conduct multi-location trials to identify lines with the desired agronomic, tuber quality and processing traits and the potential to reduce acrylamide in the near term.

Fry market – This sub objective builds on the

National Fry Processor trials. The goal of this industry-funded trial is to establish the range of reducing sugars and asn in tubers and acrylamide in fries achievable with current germplasm. **Method.** Industry-funded variety trials began in 2011 with 75 advanced clones and varieties from public breeding programs throughout the US. SCRI expanded these trials from three to five locations in 2012. Each line will be grown in non-replicated 15-hill plots at five locations (Tier 2) along with the standard French fry processing varieties Russet Burbank and Ranger Russet as benchmarks. 2013 Trials in WA, ID and ND are funded by industry and those in WI and ME will be funded by SCRI. Harvested tubers will be graded using USDA standards and samples will be sent to the USDA Sugarbeet and Potato Worksite at East Grand Forks (EGF) to quantify tuber glucose, fructose, sucrose and asn, at 1 and 3 months after harvest. Samples will be fried using standard protocols and fry color and defects evaluated. Acrylamide content in fry samples will be quantified after processing. Goals developed by industry for agronomic, tuber quality and processing traits are listed in Table 1. **Outcome.** The top 10 national performers and 5-10 regional top performers that meet targets for both raw and finished product quality will be placed in Tier 3 trials at the same five locations. The lines to be advanced will be selected by the advisory committee in consultation with the potato breeders. Tier 3 trials are described in Objective 5.

Chip market - This project will build upon the on-going industry-funded National Chip Processor Trial (NCPT) that was first conducted in 2010. The NCPT screens early-, mid- and advanced-generation clones across twelve locations in the US with the goal of identifying chipping varieties that can replace two standard cultivars, Atlantic and Snowden. These cultivars are widely used, but have major shortcomings in both production and processing. **Method.** The 2013 NCPT Tier 2 has replicated trials with up to 70 clones, with two 15-hill replications per clones, at 12 trial sites). Yield, tuber size, plant characteristics, chip color, specific gravity, and

tuber defects will be measured at each site. **Outcome.** The top performing Tier 2 clones are advanced into Tier 3 and commercial scale trials described in Objective 5.

Critical need. These industry-funded efforts will be augmented by this SCRI project, which adds a crucial focus on acrylamide reduction as part of the variety selection and development process. **Method.** Tier 2 clones and standard cultivars will be evaluated for chip color, chip defects, reducing sugars, scab resistance, potato virus Y resistance and other characteristics. Tuber samples from the southern test locations will be collected at harvest, while samples from northern locations will be collected at harvest and at two times out of storage. The data from this project will be used to help identify clones with greatest potential for meeting agronomic and quality criteria for chip growers and processors. Best clones will be evaluated for asn, acrylamide and consumer attributes in Tier 3 trials.

Critical need. Data collected in multi-location trials must be made accessible to industry.

Method. Data from Tier 1 to Tier 3 evaluations will be combined in a database that contains breeding parents, performance measures and overall merit for each line at each location in each year. The database developed for the NCPT (<http://potatoes.ncsu.edu/ncbtsrch.php>) by Co-PD Yenko will be used as the starting point. This will be adapted and utilized for the NFPT with funding from USDA SCRI. **Outcome.** This database will guide the advisory committee and potato breeders in selecting lines for advancement in the near term and will be used by extension specialists in developing production profiles for growers and processors in subsequent years.

2.3.2. Sub-objective 1b. Evaluate consumer attributes of trial lines to determine potential for commercialization.

Critical need. New varieties must be acceptable to consumers in order to succeed. Consumer evaluations cannot wait until a new variety has completed agronomic and process quality testing.

Fry market - Method. Samples from the most promising Tier 2 clones will be processed into standard fries and evaluated by a trained sensory panel for appearance, texture and taste at the J.R. Simplot pilot plant in Caldwell ID. **Outcome.** Genotypes that best meet agronomic and consumer attribute criteria will be promoted for multiplication of seed tubers. These lines will be evaluated in further agronomic and large-scale pre-commercial trials in cooperation with growers, processing and chipping plants (Objective 5).

Chip market - Method. As part of the industry-funded NCPT, chips made from Tier 2 trial samples will be evaluated by trained personnel at each respective trial site. Chips of select lines processed at commercial plants will be assessed by Co-PD Jansky. **Outcome.** The top 15-20 clones with acceptable or superior consumer attributes will be entered into Tier 3 trials at up to nine trial sites, depending regional adaptability, as described in Objective 5.

2.3.3. Sub-objective 1c. Explore new technologies for rapid measurement of tuber reducing sugars and asparagine and end product acrylamide content. Yenko, Truong.

Critical need. Acrylamide is formed primarily from reducing sugars and the amino acid asn. The HPLC, LC-MS and GC-MS methods currently used to measure these constituents are very expensive, with commercial laboratories often charging over \$200 per sample and taking more than two weeks to quantify asn and acrylamide in processed potato samples. Developing more efficient, cost effective methods to quantify reducing sugars, asn and acrylamide in potato samples would represent a significant step forward for the processing industry and it would enable the evaluation of a wider range of breeding materials, facilitating potato breeding efforts.

Method. Near-infrared spectroscopy (NIRS) is an established technique for rapidly analyzing the

composition of complex foods. NIRS is fast and suited to online, contact-free monitoring and has been used successfully to measure acrylamide in potato chips. Current methods have not been optimized to meet the higher throughput needs of potato breeders or the high sensitivity needs of the processing industry. To date, we can accurately quantify sucrose, glucose, fructose, total starch, and anthocyanin content in a wide range of sweet potato storage root samples using a FOSS XDS Rapid Content Analyzer FT Near Infrared Spectrometer. This work was conducted in collaboration with the Dr. Thomas Zum Felde, an NIRS expert at The International Potato Center, Lima, Peru, in the Center's Quality and Nutrition Laboratory. Chip and fry samples of various potato genotypes containing a range of reducing sugar and asn contents will be analyzed for acrylamide content in multiple objectives in this proposal. Replicate sub-samples will be scanned in the diffuse reflectance mode (400-2500 nm) of the NIR spectrometer. The mean NIR spectra of the analyzed samples will be modeled against acrylamide data using partial least square regression, and the regression models will be validated using standard NIR procedures. We propose to optimize NIR methods for processed potato products with the goal of achieving an accuracy of +/-20% in the range of 0-500 ppb acrylamide. **Outcome.** Potato breeders and industry allies will be able to reliably and efficiently screen larger numbers of materials for acrylamide.

2.4 Objective 2. Establish quantitative goals for tuber reducing sugars and asparagine in next generation potato varieties. Jiang, Bethke, Gupta, Jansky, Olsen, Navarre.

Critical needs. Potato has not been bred for low acrylamide in end products. Specific breeding targets for acrylamide precursors in potato tubers and relationships between acrylamide in finished products and acrylamide precursors must be defined precisely. **Objectives.** We will develop lines from elite cultivars that will allow us to precisely quantify the impact of low reducing sugars and low asn in raw potato tubers on the final acrylamide concentration in French fries and potato chips.

2.4.1. Sub-objective 2a. Create lines of Russet Burbank, Atlantic and MegaChip having a range of tuber reducing sugars and asn.

Method. The vacuolar invertase gene (*Vinv*) has been silenced in chipping varieties Atlantic and MegaChip and processing variety Russet Burbank by Co-PD Jiang. *Vinv*-silenced lines of Atlantic, MegaChip and Russet Burbank were transformed with RNAi-silencing constructs that target conserved regions of the two *Asy* genes. Additional plants of each cultivar were transformed using double-silencing RNAi constructs designed to silence both the *Vinv* and *Asy* genes. **Outcome.** Based on prior work, we expect that these double silencing lines will have very low to low *Vinv* gene expression and low to moderate levels of *Asy* gene expression and we will verify this. Tubers from these plants are expected to have very low to low reducing sugars compared to current cultivars and a wide range of asn. When will also confirm this in greenhouse and field grown tubers.

2.4.2. Sub-objective 2b. Phenotypically and physiologically characterize gene silencing lines

Critical need. Plants producing tubers with low acrylamide forming potential must retain exceptional agronomic traits. Any potential negative effects resulting from low invertase expression and low tuber asn must be established prior to undertaking large-scale breeding

efforts. **Method.** *Asy/Vinv* double silencing lines will be multiplied rapidly using standard in vitro tissue culture methods and greenhouse-based propagation. In vitro plants and tubers will be distributed to Co-PD Bethke as they become available for phenotypic and physiological characterization. **Growth and development.** Plants will be grown in both the field to examine whether double silencing introduces negative impacts on vine or tuber development, tuber specific gravity, yield, dormancy or rates of emergence. We have been unable to detect negative impacts of silencing the vacuolar invertase gene in our previous research. **Tuber characteristics.** Tuber reducing sugar contents are strongly dependent on temperature in storage, storage duration and genotype. A limited number of tubers from greenhouse trials in year 2 will be available for quantification of tuber reducing sugars following storage for 2 months at 5.5°C. In years 3 and 4, tubers of 6 select silencing lines with low acrylamide forming potential and as wide a range of tuber asn and reducing sugars as possible will be stored at 12°C for one month after harvest to assure complete wound healing and preconditioning and then will be cooled to storage temperatures of 9, 7 or 5.5°C. Tubers will be assayed by HPLC for the amount of glucose, fructose, sucrose, and the predominant organic acids citrate and malate before processing on Jan 1, March 1, May 1 and July 1. Processed fries and potato chips will be evaluated for color and for acrylamide content using industry standard procedures. Tuber asn is not expected to change substantially in storage and will be quantified in the January and July samples. Freeze-dried samples from the March and May evaluation dates will be retained and asn will be quantified if these data indicate that asn changes in storage. **Outcome.** Data from this unique set of materials will reveal the relative impacts of asn (in the background of low reducing sugar) and reducing sugars (in the background of low asn) on acrylamide in end products. We have already demonstrated that the acrylamide content of potato chips from cold-stored *Vinv* silencing lines can be reduced by >90%. Similarly, the acrylamide content of fries processed from *Asy* silencing lines can be reduced by >90%. Thus, acrylamide content in double silencing lines may reach the threshold of 20 ppb, which is considered undetectable. Data will also show whether having very low invertase and asparagine synthetase activities in a single plant will result in unexpected, negative consequences that would prevent commercialization.

2.4.3. Sub-objective 2c. Establish quantitative relationships between tuber composition and acrylamide in end products.

Critical need. The potato industry is not able to use raw product composition to accurately predict acrylamide content in finished chips, fries and other processed products. Existing acrylamide assays are costly and analysis in commercial labs can take up to two weeks. **Method.** Tubers with a range of asn and reducing sugars, water content, specific gravity and organic acids will be generated in these experiments. Acrylamide in finished products will also be quantified. Because specific antioxidants have been shown to reduce acrylamide formation in many cases, and because several ongoing breeding efforts are directed at increasing the antioxidant content of potato, antioxidant profiles in select GM and non-GM tubers will be quantified using LCMS methods that are routine in the Navarre lab. This will establish to what extent various tuber components can mitigate acrylamide formation and contribute to ALARA levels; such mechanisms may be particularly effective in genotypes having reduced acrylamide formation due to lesser amounts of acrylamide precursors. These data, in conjunction with those from Objective 1, will be used to develop a predictive model for acrylamide content in finished products based on the composition of raw product. The approach will include linear and non-linear regression and principle component analysis. Existing data on acrylamide in potato

products indicate that there is considerable variation around the mean. Estimates for this variation will be incorporated into the model to increase its utility. Unlike previous efforts, this analysis will focus only on tubers with low asn and low reducing sugars and results will not be skewed by high but irrelevant amounts of these compounds. **Outcome.** A model will be developed that predicts acrylamide formation and likely end product quality based on raw product composition.

2.5 Objective 3. Identify and validate molecular markers associated with key agronomic and tuber quality traits that can increase the efficiency of potato breeding programs.

Critical needs: Potato breeding programs need molecular markers to select progeny and breeding parents more rapidly and efficiently. This will decrease the time and resources needed to develop new varieties with a high occurrence of favorable traits. Phenotypic data are needed to establish and confirm trait associations for the SNP markers developed in the SolCAP project. The range of asn in US germplasm and heritability of asn in potato needs to be defined.

2.5.1 Sub-objective 3a. Identify SNP markers for high value traits in russet potatoes. Novy, Bethke, Jansky. **Critical need.** High utility SNP markers that associate in predictable ways with tuber quality traits are needed for potato breeding. SNP markers must be validated to confirm associations between genotype (SNPs) and phenotype (tuber sugar content, asn) over a range of production environments, storage times and temperatures as well as across mapping populations. **Method.** The USDA/AFRI coordinated agricultural project, SolCAP, has developed and validated 8,303 high-throughput, genome-wide SNP markers for potato. These SNP markers can be used to query the genomes of elite germplasm. A SNP-based genotyping array has been designed to survey the majority of the potato genome with a focus on candidate genes involved in carbohydrate metabolism. An effort was made to target select carbohydrate biosynthesis genes with as many SNPs as possible. For example, 114 SNPs are within 12 carbohydrate-associated candidate genes, with sucrose phosphate synthase and acid invertase having 20 and 16 SNPs, respectively. Initial studies through SolCAP are looking at linkages between SNP markers and tuber reducing sugar and sucrose concentrations in the Premier Russet x Rio Grande Russet mapping population out of 10°C storage. The trait-associated SNP markers identified will be based on a limited amount of phenotypic data, however, and many will have little utility when applied more generally. To identify those SNP markers with robust utility, a subset of 43 clones and their parents from the SolCAP Premier Russet x Rio Grande Russet mapping population is being grown in ID and MI in 2012 and 2013, and stored at final set points of 4, 6, 8, and 10°C after one month of preconditioning at 12°C. Clones were selected from, and representative of, the original 199 clone mapping population based on data for tuber concentrations of sucrose, glucose, and fructose. Freeze dried tuber tissue samples are being used for determinations of tuber sucrose, glucose, fructose and asn. Processed samples will be used to assess fry color and acrylamide content. Metabolic pathways for reducing sugar formation are well characterized and available data suggest that tuber sugar formation is controlled by a small number of key genetic loci. The use of 50 lines from the original SolCAP mapping population will allow us to determine more fully the effects of environment on marker stability without sacrificing essential genetic variability for these traits. **Outcomes.** Increased confidence in SNP markers associated with desirable tuber carbohydrate composition and candidate genetic markers for breeders to apply in

marker-assisted selection.

2.5.2. Sub-objective 3b. Combine the phenotypic evaluation of a mapping population with the genotype data generated by SolCAP to identify marker-trait associations for genes with large effects on market traits. Douches, Bethke, Jansky. The SNP markers developed in SolCAP are being used to genotype a germplasm panel composed of 225 elite potato varieties and breeding lines. Replicated phenotypic data are being collected from three environments (NY, WI and OR) for two years. Association mapping for chip-processing traits such as specific gravity, chip color and tuber reducing sugar content will be conducted and SNP markers linked to these traits will be identified.

Critical need. To rapidly and efficiently reduce the acrylamide forming potential of next generation potato varieties, molecular markers associated with tuber reducing sugars and asn content and end product acrylamide are required. **Method.** This SCRI effort will extend the association mapping studies in SolCAP to include tuber asn content. To confirm the results of association mapping, we have chosen a mapping population that is segregating for important processing and disease resistance traits. The parents chosen (W2130-3 and Kalkaska) are chip-processing potatoes that differ for key traits such as chip color, reducing sugar content, acid invertase activity, starch content and common scab resistance. We expect many of these traits to segregate in the progeny. Moreover, one parent of W2130-3 is S440, a tetraploid clone that contains *S. tarijense* germplasm and is noted for its resistance to cold-induced sweetening. All these traits are important to the chip-processing industry. The W2130-3 x Kalkaska cross is also expected to produce advanced breeding lines with commercial potential that combine yield, tuber appearance, low reducing sugar and high starch levels in tubers, and scab resistance. We have examined asn concentration in tubers the progeny and they are segregating for asn concentration. We are now positioned to expand our analysis and examine tuber reducing sugar, tuber asn, and chip acrylamide content in this mapping population. This mapping population complements the russet potato mapping population (Premier Russet x Rio Grande Russet) described in section 2.5.1. Therefore, we expect that the QTL analysis in the chip-processing mapping population will complement the russet potato mapping population since chip processing potatoes require a more stringent maintenance of low reducing sugars than russet processing potatoes. Mapping QTL in this chip-processing population may uncover unique or complementary QTL not expressed in the russet mapping population, especially with its pedigree containing *S. tarijense*, which is not represented in the russet mapping population. **Method.** Replicated trials are planned for the 2012 and 2013 field seasons. Tubers will be collected at harvest for chip processing trait analysis as described above. QTL analysis based on candidate gene and genome-wide SNP will be conducted. Identified SNPs linked to QTL (for the traits above) will be used for validation of marker-assisted-breeding prior to subsequent use by the potato breeding community. **Outcomes.** QTL for processing traits will be identified. SNP markers that are linked to processing traits can then be used by breeders for marker-assisted selection of lower reducing sugar and asn levels in the progeny. Marker selection can be used during early generation selection to more efficiently identify the promising selections with the processing characteristics (low reducing sugars, low asparagine, and high starch) demanded by the chip-processing industry. We also expect to select advanced breeding lines with commercial potential.

2.5.3. Sub-objective 3c. Determine the range of tuber asparagine in US potato germplasm and the heritability of tuber asparagine.

Critical need. Potato breeding activities must include selection for low acrylamide precursors, but breeding for low tuber asn has not been done in US potato breeding programs and there is very limited information on asn levels in parental clones or the heritability of tuber asn. Potato breeders need data on tuber asn to identify parents that can be used in crossing programs for producing low asn/low acrylamide processing cultivars. **Method.** Part of this objective will be accomplished for fry varieties in the industry-funded NFPT. Data on asn in chip clones will be generated for Tier 3 materials being evaluated in the NCPT. Tuber asn content for the germplasm panel composed of 225 elite potato varieties and breeding lines and the russet mapping population being genotyped by SolCAP was determined following production in WI to establish the range of tuber asn in US cultivars. This will generate data that can be used for association mapping of tuber asn. Breeding programs from the US will have the opportunity to request that parental clones be added to a central grow-out site (MI) where each clone will be grown along with industry standard varieties. Tubers from this grow-out site will be collected and analyzed for asn content. **Outcome.** The data will identify clones and lineages that have reduced tuber asn contents and will assure that each breeding program can conduct informed crosses for reducing acrylamide formation in processed potatoes. Data on tuber sugars and asn will be made available to the potato breeding community and industry partners through the database generated for the variety trials.

Critical need. Potato products low in acrylamide must be made from tubers with low amounts of asn, but the heritability of tuber asn is not known and the expected rate of genetic gain achievable through breeding is unknown. **Method.** Segregation of asn in tubers has been observed in a limited evaluation of progeny from a segregating population produced by crossing the cultivar Kalkaska and the advanced line W2310-3. Funds from SolCAP are being used to genotype the population using SNP markers. Heritability of tuber asn and a preliminary assessment of the potential for genetic improvement will be evaluated by further generating mapping populations in which lines producing low asn tubers are crossed to each other and lines producing high tuber asn are crossed and the tuber asn content in progeny quantified. **Outcome.** A strategy to generate low asn lines using conventional breeding /marker-assisted selection.

2.6 Objective 4. Quantify the economic value and risks associated with introducing new potato varieties that have low acrylamide forming potential.

Critical need. Economic valuations are needed for new varieties with low acrylamide forming potential relative to current benchmarks so that industry can make sound business decisions.

Varieties that meet acrylamide goals must have acceptable agronomic, processing and consumer acceptance characteristics. Field evaluations, acrylamide analyses, processing test runs and other evaluations conducted in Objectives 1 and 5 will generate variety performance data. The suite of performance changes observed needs to be considered as a whole, as no new variety is likely to improve all product characteristics. Tradeoffs exist—a new variety may reduce acrylamide, but this benefit may need to be balanced by cost differences for growing, processing, or selling the variety. A new variety may require more costly agronomic management practices to maintain raw product quality, have processing characteristics that reduce recovery, or have greater

difficulty in the market due to consumer concerns beyond acrylamide. Objective 4 uses net product value to integrate over all these product characteristics to quantitatively determine the net effect of new low acrylamide varieties across the whole processed potato system, including any risk changes these varieties bring to producers, processors/distributors, or consumers and marketing. Following the three primary sectors of the processed potato industry (Fig 1), Objective 4 examines the effect of new processing potato varieties for the production sector, the distribution and processing sector, and the consumer and markets sector, with a different economist leading each.

2.6.1. Sub-objective 4a. Estimate the net value and risks for growers of using new potato varieties and/or production methods to reduce acrylamide in finished products. Mitchell.

Methods. The net value and risk of new field and storage practices will be estimated using a series of hierarchical models that link production practices to the statistical distributions of product quality traits (e.g., tuber size, tuber density) and then to final product value, both out of the field and out of storage of different durations. Hierarchical models reduce complex processes to a series of linked conditional and marginal probability distributions. The parameters of one conditional distribution depend on another random variable with its own parameters that depend on another random variable, and so on, until reaching final unconditional distributions. For example, the hierarchical model for tuber sugar content will involve estimating a probability distribution for tuber sugar content with parameters conditional on harvest timing relative to peak tuber maturity, storage temperature, etc. Conditional densities will be estimated via maximum likelihood using existing data from project collaborators and new data gathered through this project.

Quantitative measures. By estimating the conditional probability distribution of the variable of interest, the hierarchical model allows analysis of how management affects the distribution of tuber sugar content. In other words, management changes the mean and variance of tuber sugar content, but the actual sugar content realized is still random. Thus, the analysis will incorporate risk—how management practices affect the expected value (average) of returns, as well as their variance. Probabilistic analysis, such as how harvest timing or other practices affect the probability of tuber sugar content (or some other product trait), can be included to establish a risk threshold. Risk is incorporated by using Monte Carlo analysis to draw from the conditional distributions linked in the hierarchy. See Mitchell and Hutchison for a discussion of risk analyses for insect-crop systems, but the general principles apply here as well.

Mitchell has developed hierarchical models for insect-crop systems and has begun developing similar models for potato management. The cost of adopting these new field and storage practices is an important aspect of the economic analysis needed to determine the net benefit to farmers and processors. Cost data for specific practices are being developed by communicating with input suppliers, growers and processors. Mitchell has developed similar cost estimates for other cropping practices and other crops as part of his Extension program. Mitchell and Bussan is a preliminary version of this approach. Data from experimental plots were used to estimate conditional probability densities for the effect of deep tillage and planting density on the size distribution of harvested tubers. These were then combined with cost data for deep tillage and seed potato prices in a Monte Carlo analysis to examine the net economic value and changes in grower income risk from managing tuber size using tillage and planting density.

Outcome. Value and risk assessment models for field and storage management techniques that can be used to optimize crop quality, increase grower profitability and assess the value of new varieties.

2.6.2. Sub-objective 4b. Quantify the net value and risk to the potato processing industry associated with introducing new varieties and management practices. Guenther.

Methods. Processing value indexes for potatoes will be developed to provide a variety evaluation tool that includes economics and risk. Variables included in the indexes are:

- Tuber attributes targeted in Sub-objective 1a that affect processing costs
- Attributes that affect raw product costs identified in Objective 4a
- Differences in product value discovered in Objective 4c
- Ranges of attribute values that affect risk
- Costs of identity preservation (IP) practices for GM varieties

Interviews with potato processing experts will quantify the impacts on costs and revenue of the attributes in the indexes.

Quantitative measures. The frozen processing value index and the chip processing value index will be on a scale from 0 to 100. The index number will be used to compare the relative processing value of potato varieties. It will not give an estimate of exact processing costs because of variable expenses, such as cooking oil and energy that are not included in the analysis.

Processing value index numbers will be calculated for each variety that is currently used in potato processing. Those varieties will serve as a base to which new varieties will be compared.

Outcome. The final product will be a list of processing varieties and promising new lines, each with a potato processing index number. This rating will aid the potato industry in making informed variety selection decisions and thereby enhance potato industry profitability and sustainability.

2.6.3. Sub-objective 4c. Analyze consumer perceptions regarding processed potato products including acrylamide content and use of GM tubers. Huffman.

Methods. The new laboratory auction methods of Rousu et al. will be used to examine consumer acceptance and willingness to pay for low acrylamide potato products. An independent survey agency will identify a random sample of 300 consumers 18-65 years of age in three U.S. cities (one in the West, Central and East) to participate in experiments. Sessions will take about 90 minutes. Participants will be paid \$65, with 18 total sessions (six at each site). Three potato products will be used to allow for consumer preference diversity: a 5 pound bag of potatoes, a one-pound package of potato chips (high acrylamide product) and a loaf of potato bread (low acrylamide product). Following Rousu et al., products will bear food labels, with information about acrylamide and genetic modification used as treatments in the auction setting.

Consumers will come to a laboratory or classroom and be randomly assigned to one of two concurrent sessions. Participants will first complete a consent form, then a questionnaire for socio-demographic information, food preferences, and technology preferences. Next, they will engage in two practice rounds of bidding to gain familiarity with the auction mechanism—a sealed bid, private value, random n^{th} price auction. Shogren et al. have shown that this auction mechanism better reveals willingness to pay for new goods than other mechanisms. The session monitor collects product bids and ranks them from highest (1) to lowest (N), then draws a random number n from 1 to (N-1) for the auction winners. For example, if $N = 15$ and $n = 5$, then

the four highest bidders pay the 5th highest price. Hence, each bidder has a probability of being a “winner,” which increases the sincerity of bidding, especially for those with low values. Because acrylamide and genetic modification raise complex scientific and social issues, four types of information will be used as treatments: (1) no information baseline, (2) a “food and biotech industry” perspective (pro-biotech, neutral on acrylamide), (3) “environmental group” perspective (negative on GMOs and high acrylamide levels), and (4) independent, 3rd party perspective (objective given state of information). Some sessions will receive no information, and other sessions will receive one, two or three types of information. Information treatments will be randomly assigned to sessions so that unobserved participant effects are uncorrelated with treatments.

Participants will make bids on three products in four bidding rounds, each displaying information on a plain label with product name, weight, and date: (1) standard acrylamide level, (2) California acrylamide warning “cooked potatoes that have been browned, such as French fries, baked potatoes, and potato chips, contain acrylamide, a chemical known to the state of California to cause cancer,” (3) statement that “product contains reduced acrylamide levels due to conventional breeding methods,” and (4) statement that “product contains reduced acrylamide levels due to intragenic GM methods.” Label ordering will be randomized in each session to minimize sequencing effects. Only one round will involve exchanging money for products, so each participant will purchase at most one unit of each auctioned product. Bid-price data collected from consumers will be analyzed using an econometric model of bid prices as a function of socio-demographic attributes, food and technology preferences, and label and information treatments. Bid prices will be pooled across products with product fixed-effects and across individuals using observables to control for participant heterogeneity. We hypothesize that consumers are willing to pay significantly more for new low-acrylamide products, especially chips, relative to similar plain-label products. We hypothesize that intragenic GM low-acrylamide products will have higher value than products with standard acrylamide levels. We hypothesize that the California acrylamide warning to have a large negative effect on willingness to pay for chips and it may also have negative spillover effects on other bid prices placed after seeing the California warning. **Outcome.** These data will provide a quantitative assessment of consumer preference for potato products with varying amounts of acrylamide.

2.7 Objective 5. Implement a rapid method for deploying potato varieties that exceed expectations for quality throughout the potato industry and consistently yield chip and processed potato products with acrylamide contents lower than current benchmarks.

Critical need. Adoption of new potato varieties has been incredibly challenging for the potato processing and chip industries, yet new varieties are essential for optimizing product quality and reducing acrylamide. *This proposal objective will directly address the most serious problems associated with new variety adoption including:*

1. Inadequate participation of processors and end users in the variety development process to identify problems with consumer attributes early in the selection process.
2. A lack of in-depth recommendations for cultural and storage management and information on disease susceptibility rankings for new varieties in the field and in storage.

3. Reluctance of seed producers or breeding programs to assume the financial risks of ramping up virus free seed production for commercial scale trials of advanced lines.
4. Variety weaknesses are not uncovered until after large-scale production and storage evaluations begin.
5. Ineffective or incomplete communication of trial results to growers and processors.

Approach. *Extensive, large-scale evaluation is the most effective tool available for promoting variety adoption and assuring long-term variety success.* Efficient large-scale evaluation of promising new varieties requires the coordinated action of researchers, extension specialists and industry personnel having a range of expertise. Materials identified by the advisory committee and potato breeders in Objective 1 will be submitted for cleanup and elimination of disease in seed. The most promising advanced potato clones will be given to potato agronomists and production specialists across the country to evaluate agronomic traits and provide production data to potato growers and processors. Those varieties with the greatest potential for commercialization will be submitted for commercial-scale Tier 4 trialing to develop agronomic and storage practices with focus on meeting all quality attributes identified earlier, as well as to assess stress and pest tolerance, and the ability of tubers to maintain quality in bulk storage.

2.7.1. Sub-objective 5a. Establish standardized agronomic trials for evaluating tuber productivity, quality, and assessing finished product quality.

Critical Needs. Extension specialists, processors, chip companies, and growers need to become familiar with Tier 3 chip and processing lines. This promotes industry participation in the variety development process and provides agronomists and extension specialists the opportunity to identify breeding lines with the greatest fit for local production and processing systems.

Approach. Breeding lines with the greatest potential based on evaluations in Objective 1 are submitted for seed cleanup and production. Replicated research trials will be conducted at multiple locations nationally to demonstrate the suitability of these breeding lines for meeting defined tuber quality and acrylamide specifications. Tier 3 trials are designed to assess the stability of quality traits under various production and storage regimes described in sub objectives 5b and 5c. These data will provide opportunities for growers and processors to evaluate materials regionally and locally, as trials will be conducted on potato grower farms and raw potatoes will be supplied to local chip and process facilities for evaluation of end products.

Method. Fry market. The top performing Tier 2 clones each year will advance to SCRI-funded Tier 3 testing during the following year. Each clone will be evaluated in replicated two-row plots, 15 m long, with 4 replications in ID, ME, OR, WA and WI. Agronomic and acrylamide data will be collected as in Tier 2 trials. Processing quality evaluations at the JR Simplot pilot plant will include a more extensive bank of color, uniformity, and taste panel criteria of critical importance to the processing industry (see 2.3.2). These additional evaluation criteria are required to establish the likelihood of industry adoption for each line. **Outcome.** Disease free seed will be produced for each line in the Tier 3 trials. Two to four top-performing clones will be identified for pre-commercial trials and processing plant evaluations in Tier 4 trials. Consumer attribute information available from objective 1 will be considered in promoting lines to Tier 4.

Method. Chip market. As part of the industry-funded NCPT, 15 to 20 chip clones will be selected from Tier 2 chip trials. Disease-free or best-available seed of each line and reference varieties will be used in replicated agronomic and processing trials conducted at 11 locations across the country. During the production year, clones will be evaluated for susceptibility to soil

borne and foliar diseases. At harvest, yield and quality attributes will be evaluated as described earlier. In addition, tubers will be evaluated for tuber diseases. After harvest, each clone will be evaluated for sugar and asn content and processed for chip color and defects. The acrylamide content of chip samples will be quantified. Bulk storage trials will be conducted at MI, ND, ID, and WI. Varieties will be evaluated for sugar content, chip color, defects, and acrylamide after 4 and 8 months of storage at 9°C. **Outcome.** Once industry partners are satisfied that small-scale samples and agronomic performance meet their quality criteria, seed supplies will be expanded to allow Tier 4 evaluations including large-scale runs through chipping plants facilitated by the USPB sponsored “fast track” seed program.

2.7.2 Sub-objective 5b. Develop production profiles and maturity indices for assessing retention of low acrylamide forming characteristics in chip and frozen processing lines.

Knowles, Pavek, Rosen, Porter, Bussan, Thornton.

Tier 4 lines will be evaluated experimentally to determine agronomic best management practices to improve likelihood of success in commercial trials. Tuber maturity has a large effect on processing quality at harvest and the retention of processing attributes during storage.

Environmental conditions during production interact with management practices to dictate the maturity of tubers at harvest affecting storability of processing potatoes. Tuber maturity is defined by skin set (physical maturity), dry matter content (physiological maturity) and sucrose content (chemical maturity). Tubers have the longest storage life for processing if harvested at PM (physiological and chemical maturity). Cultural and nitrogen management affect tuber protein and asn levels and the timing of PM. Advanced clones selected for low acrylamide forming potential must either possess resistance to N-induced variation in tuber PM, or management recommendations need to be developed to harvest at PM so that process quality and low acrylamide forming potential are retained in storage.

Critical Need. The interaction of climate and nitrogen management on attainment of tuber PM and its subsequent effects on the ability to retain low tuber asn and reducing sugar contents and low acrylamide forming potential during prolonged storage needs to be understood for the chip and process clones promoted into the commercial scale trials

Approach: The effects of tuber PM on retention of tuber processing quality will be evaluated for the Tier 4 chip and fry clones. Varying the rate of N applied will produce tubers that mature at different rates. N will be applied at 0, 50, 100, and 150% of the rate recommended for standard cultivars. Process potatoes will be grown at WA, ME, and ID and chip potato clones will be grown at ME, MN and WI. Data on foliar and tuber growth and tuber starch deposition will be collected at 15-day intervals. Modeling foliar and tuber development over time and in relation to accumulated degree-days from planting will reveal N nutrition affects at critical growth-stages (plant establishment, tuberization, bulking, maturation, foliar senescence) and will facilitate estimation of tuber PM attainment for each clone. Harvest indices and bulking rates will be calculated and tuber samples will be analyzed for sucrose, glucose, fructose, asn, and dry matter through the season. Tuber PM will be estimated as the average of days after planting (DAP) to minimum tuber sucrose, maximum specific gravity, and maximum tuber yield. Clone-specific indices of PM will be developed for use by industry based on average DAP and degree days to reach PM, extent of vine senescence at PM, and relative tuber carbohydrate levels (stem/bud reducing sugar ratio; sucrose concentration). Tubers will also be analyzed for N, protein, and amino acids (including asn) to assess nutritional value and the potential for acrylamide formation.

The ability of these cultivars to maintain processing quality and nutritional value will be assessed during the storage phase of this study. Tubers from the final harvest will be held at 12°C for two to four weeks and then stored at 5.5, 7, and 9°C. Sprout inhibitor will be applied in Nov/Dec using industry standard rates. Tubers will be sampled for sucrose, reducing sugar and fry color analyses at 30- to 60-day intervals through June (~250 days). Changes in tuber sugars and processing quality will be correlated with differences in PM. Quantification of asn and reducing sugars will facilitate estimation of changes in acrylamide forming potential. **Outcome.** The results will demonstrate the sensitivity of Tier 4 clones to in-season and post-season management practices with respect to processing and acrylamide forming potential. Development of guidelines for specific agronomic practices will decrease risks to growers producing new varieties. Maturity indices will be used to determine the optimum time to harvest and will maximize processing quality and low acrylamide forming potential during storage.

2.7.3. Sub-objective 5c. Develop storage profiles that optimize management of new varieties on a regional basis. Olsen, Porter and Bussan.

Critical Need. Potato clones differ in their sensitivity to the storage environment and optimal storage management practices must be developed for each new variety. As a result, each variety requires specific storage management protocols for suppression of sprouts, disease management, and optimization of temperature to manage sugars and fry color and minimize acrylamide. Development of storage management recommendations for new varieties improves potential for success in commercialization of new varieties. **Methods.** Tier 4 potato clones evaluated in Objective 5b will be evaluated for storage characteristics. Storage trials will be conducted at ID, WI, and ME to rapidly assess the influence of production environment, crop maturity, and genotype on tuber quality in storage, sprouting, disease development and shrink. Each clone will be preconditioned at 12°C until sugars reach acceptable levels for chipping or processing and then cooled to 5.5, 7.5 and 10 C Trials will evaluate: preconditioning duration required; optimal long-term storage temperature; and maximum length of time that tubers retain high processing quality. Sprouting and tuber sugars will be evaluated monthly on each variety. Fry and chip color will be evaluated every month and acrylamide will be evaluated after 4 and 8 months of storage. **Outcomes.** Variety specific storage management recommendations will be produced that will help storage managers minimize tuber weight loss, disease incidence, sprouting and cold induced sweetening. Growers producing new varieties in commercial trials will be given guidance on optimal storage conditions that maximize end product quality and minimize acrylamide.

2.7.4 Objective 5d. Conduct on-farm commercial production trials with growers. Bussan, Olsen, Porter, Pavsek.

Critical need. The largest challenge faced by the potato industry is the financial risk associated with producing enough potato seed to allow for commercial evaluation. Large-scale, multi-location, mechanical harvesting, handling, and processing runs are required to assure that quality can meet industry standards for bruise free, color and texture, and defects during commercial-scale processing. Individual breeding programs cannot afford to conduct evaluations on this scale while end users require commercial runs to assess quality risks of new varieties. This delays adoption of promising new varieties since large scale commercial trials are required to demonstrate the value of new varieties to industry. **Methods.** In year 3 and 4, two chip and two processed potato varieties will be evaluated in commercial-scale trials for a total of 8 evaluations. This will require production of up to 10,000 kg of virus-free seed tubers for each variety through

the Tier 4 Fast-Track program. Two to four growers will receive 2,000 to 4,000 kg of seed for commercial production with the goal of harvesting 100,000 kg at each location. The harvested crop will be stored in simulated commercial storages and processed through a commercial chip or processing plant to evaluate performance. Potatoes will be grown and stored in commercial-scale trials based on methods developed through sections 2.7.2 and 2.7.3.

2.8 Outreach Plan.

Establish a strategy for deploying new varieties in cooperation with growers, grower organizations, seed producers, processors, distributors and the food service industry.

- Establish effective channels of communication among stakeholders and researchers.
- Establish procedures for evaluating research progress and implementing adjustments when and where needed.
- Establish a database of key performance parameters for varieties and breeding lines.
- Implement a process where lines identified by growers, processors and researchers can be evaluated on a commercial scale without substantial risk to individual growers.

In conjunction with our industry partners, this project will identify highly promising lines, produce virus-free seed potatoes, grow potatoes of new lines in large volumes, and conduct commercial runs of processed and chip potatoes. **Outcome.** Promising fry and chip processing lines were identified in 2011-2012 for production of certified seed. Evaluation of these lines will occur on a commercial scale to rigorously assess potential strengths and weaknesses of each.

Critical Need. Information transfer on variety performance over climates and management systems is necessary to allow optimal production of new varieties. Many new potato varieties fail commercial tests due to poor communication of variety characteristics to growers and industry. Outreach efforts occur throughout the research and extension activities proposed, as ongoing engagement of stakeholders in the project is a vital component of this proposal.

Approach: Continue to involve food processors and end users in the variety development process at all stages so that only varieties shown to have superior consumer attributes in Sub-objective 1a are advanced for large-scale trials in Objective 5 and promoted for adoption by industry. This will substantially increase the likelihood that new varieties with low acrylamide forming potential will be utilized by industry. This will be facilitated by engaging industry members in evaluation and selection processes defined in Objectives 1 and 5. On-line database will facilitate communication between breeders, agronomists, and industry participants.

Approach: Develop variety specific guidelines that describe the agronomic, processing and consumer acceptance of new varieties with low acrylamide forming potential including:

- In depth recommendations for crop production including disease and nitrogen fertility based on growth and development and PM data collected through Objectives 1a, 1b, and 5
- Storage management recommendations on preconditioning, sprout management, disease susceptibility, minimum temperature, and long-term potential through Objective 5.
- Estimated economic value and probability of return on investment over standard varieties to growers and to processor based on analyses in Objectives 4a and 4b.

This information will be publicized and distributed by team members and an extension specialist hired for this project who will have responsibility for assembling and organizing variety-specific data and making it available through a searchable web site.

Approach: Even the best varieties have weaknesses as well as strengths. Many variety weaknesses will be uncovered during the large-scale production and storage evaluations described in Objectives 5b, 5c and 5d. These need to be communicated to industry so that cultural management, handling, storage, and processing plant procedures are in-line with variety-specific needs. Industry experience over decades has shown that failure to adjust production, storage or processing practices to optimize variety performance can slow adoption of new cultivars.

Approach: Consumers, regulators and members of the potato industry need information on acrylamide including a description of which potato products contain acrylamide, how it is formed, why it is a health concern, and what steps are being taken by industry to reduce acrylamide in processed products. Materials describing this will be developed by an extension specialist hired for this project and will be freely available through the internet.

Approach: The value of new varieties with low acrylamide forming potential must be communicated to consumers and industry. The data generated in Objective 4 will be submitted to peer-reviewed journals and presented at regional and national meetings. These data will aid industry in developing marketing strategies designed to highlight the benefits of new potato varieties and will enable industry to better assess the value of variety development efforts.

The foundation of the extension and outreach program is the current extension programs of team members who have ongoing responsibilities and experience in developing directed programs. Existing programs contribute publication resources for page and printing costs of materials.

Outcomes. Specific, quantifiable outreach goals include:

- Create website with consolidated variety trial information from Objective 1 and 5. We conservatively estimate at least 3,000 annual visits by the potato industry to this website.
- Develop variety-specific field and storage management guides for each of the lines advanced to Tier 4 trials. These data will be easily accessible via the website.
- Present findings at regional field tours and workshops and highly attended winter commodity schools (i.e. the Idaho Potato Conference (~1000 attendees), Wisconsin Potato Grower Education Conference (~500 attendees), Maine Potato Conference (~200 attendees) and Washington Potato Meeting. Multiple team members speak at state potato meetings each year.
- National extension activities including presentations and workshops at national potato and horticultural conferences such as the National Potato Council Expo. At least two national presentations will be made per year to disseminate findings.
- Publish articles in trade journals such as *Spudman*, *Potato Grower*, and *The Badger Common 'Tater*, publications reaching over 10,000 subscribers and highly valued means of information dissemination to growers and end-users. At least three publications per year will be written.
- Extension bulletins and publications summarizing key research results and practical application for stakeholders. At least 4 bulletins per year will be disseminated.

- Project summaries and up to-date recommendations communicated through proceedings, newspaper and newsletter articles. At least 6 articles will be written per year.
- Provide leadership and science-based information to the community regarding specific issues of consumer quality and benefits of this research to society.

Outreach Evaluation Plan. The activities and outcomes of the outreach plan will be evaluated.

Activity evaluation. Field tours and grower/processor meetings will be evaluated by documenting the number of participants per event/activity and by surveying all participants. Participants will be asked to report on the quality, utility, and impact of the programming. Evaluation results and participant reactions will be incorporated into future meetings throughout the project to improve the overall quality and utility to the end-user.

Outcome evaluation. Several questions are important as indicators of the project outcome:

1. Did grower and processor awareness of opportunities to lower acrylamide increase?
2. Did grower and processor knowledge of new varieties and accompanying production/storage/processing strategies increase?
3. Was a variety adopted by industry? If so, what was the acreage planted?
4. What was the economic value (to the grower, processing companies, and rural economies, or end users) of the converted production acreage or stored volume?
5. What are the potential pitfalls of the variety and can they be overcome?
6. Are molecular markers developed in Objective 3 being incorporated into potato breeding programs?
7. Are the NIRS methods developed in Objective 1c used to quantify reducing sugars, asn and acrylamide in potato?
8. Are targets for acrylamide precursors developed in Objective 2 being used by breeding programs?

Awareness of the opportunities and knowledge generated in this project will be evaluated by conducting pre- and post-activity surveys during field tours and educational meetings. Grower adoption and interest in the project results will also be surveyed at this time. The shifts in raw and end product quality and economic value will be provided by the processing industry. Adoption of markers or breeding targets by potato breeders will be assessed through one-on-one interviews.

2.9 Pitfalls and limitations to proposed procedures.

Adequately describing all possible pitfalls and limitations for the wide range of research and extension goals for this project is difficult in the space available; we will rely on our research team, advisory committee, and colleagues to address them as they arise. Individual Co-PDs have experience with the methods proposed, and we do not anticipate technical difficulties in this regard. The exception to this is the NIRS optimization research described in sub-objective 1c where the research question addresses the suitability of the method for our intended need. It can be postulated that none of the germplasm currently available meets the goals we have set for product quality, consumer acceptance and acrylamide reduction. Although we consider this unlikely, the only way to determine if this is the case is to conduct evaluation efforts on the scale we have proposed. Substantial resources are devoted to analysis of acrylamide and asparagine, as sufficient resources are not available to breeding programs and state organizations. These costs may increase or decrease from our current estimates. Flexible funds are built into the budget to cover modest increases. We anticipate the need for flexibility in project direction as data become available and progress is made on the objectives proposed. Decreases in costs, like all program developments, will be communicated to the research partners, advisory committee and program manager and funding priorities adjusted to maximize value as appropriate. Physiological research and field research may be compromised by the wide array of agronomic and pest-management characteristics that must be considered with new varieties. Adverse effects on crop growth and storage resulting from weather, pests, and disease may compromise some aspects of individual trials. These events add valuable data on variety performance, however, and the multi-location design of our variety evaluation efforts makes it unlikely that data from any year will be compromised to the extent that goals cannot be met.

2.10 Hazards to personnel.

Multiple agricultural enterprises are dangerous and even small-scale potato research activities pose significant threats. Key safety concerns and mitigation includes:

Lifting hazard: Potatoes are comprised mostly of water so small volumes can be deceptively heavy. Staff, students and other personnel will be trained on proper lifting procedures to prevent back and other injuries. Lift carts, dollies, and forklifts are available to assist in handling potatoes at research facilities.

Chemical hazards: Multiple pesticides, sprout inhibitors, vine desiccants and other chemicals are used in potato production. All staff handling or applying these chemicals will have a pesticide applicator license, follow label directions, and wear personal protective equipment. Fields and facilities will be posted per US worker protection standards.

Inhalation hazard: Staff will not be allowed to enter storage bins and lockers without others being on the grounds or aware of their presence. All bins treated with sprout inhibitor or other treatments will be posted and staff notified of facility quarantine. Alarms are present on storage bins and lockers notifying managers and researchers if air quality in facility becomes unsafe.

Large equipment hazard: Staff will not be allowed to operate large equipment without proper training or certification through safety classes. All shields and protective devices on equipment will be maintained to insure project personnel safety.

Laboratory safety: All persons working in laboratories will have been instructed in procedures for safe laboratory practice, especially with regard to chemical hazards.