Since 2008, the Center for Produce Safety has funded eighty-five research programs with some of the world’s leading produce food safety scientists. As articulated in the Center’s yearly request for research proposals, we have endeavored to fund applied produce food safety research targeted to the immediate needs of all stakeholders in the produce supply chain. From that body of work, a number of key learnings have emerged that can be used to assist the produce industry in developing hazard and science-based food safety programs. This document lists some of these key findings and offers how the data might be used by those in the produce supply chain to improve their food safety programs.
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1. **Pathogen survival in commercial production environments can be variable.**

Attenuated *E. coli* O157:H7 and *Salmonella* applied directly to the soil or by a spray directly to the surface of spinach or romaine lettuce leaves dies off quickly so that it is very hard to detect after 2 days. However, pathogens may survive for longer periods when associated with organic matter. Spinach inoculated with *E. coli* O157:H7 and turned under the ground was recoverable from the soil for 100 days. When follow up experiments were performed with inoculated spinach under commercial conditions in the Salinas Valley of California (chopping the spinach and permitting it to dry out before incorporation into the soil), no *E. coli* O157:H7 was found on the second crop 27 days after planting and no *Salmonella* was detected 35 days post planting. It is thought that leaving the crop residue on top of the soil to be exposed to the sun and to become dehydrated may prevent pathogen growth and enhance pathogen die off. Similarly, attenuated *E. coli* O157:H7 inoculated into organic fertilizers and disked back into the soils, can survive for extended periods of time.

**What does this mean for you?**

- These data demonstrate that pathogens can survive in Salinas Valley production environments when associated with organic material, e.g. leaves, organic fertilizers, soils, etc. Therefore, sufficient time (27-35 days) must be scheduled to permit chopping/mowing, dehydration, incorporation and subsequent die off to manage the risk of pathogen survival in the soil and potential cross contamination to the next crop.
- If a pathogen contamination is found on a crop, rotational choices for the next crop should be considered carefully; i.e. a crop that comes to maturity in less than 27 days may not be a wise choice even if the previous contaminated crop residue was permitted to dry out prior to incorporation.
- It is important to establish time intervals for specific environments, crops and soil types as variability in pathogen survival should be expected in different production environments.
- These data clearly point out the importance of performing risk assessments on fields prior to harvest as potential contamination events closer to the time of harvest may be of a higher priority to identify than events further from harvest.
2. Buffer zones can be effective hazard management tools.
Following a natural intrusion of feral pigs into a commercial lettuce field, elevated levels generic *E. coli* were found where the pigs obviously contacted the crop, but not out beyond a 10 foot buffer zone. Wind has also been suspected as a vector for pathogen transfer. A study was conducted where leafy green plantings were situated at various distances down-wind from a cattle feedlot where the herd was known to be contaminated with *E. coli* O157:H7. The data demonstrate that *E. coli* O157:H7 can be transferred via bioaerosols and dust particles to crops at least out to 600 feet (the farthest distance tested). As distance increases away from the cattle feedlot, the frequency and level of contamination diminished. However, bioaerosol and dust particle transference is not a simple matter of distance. The density of the cattle, wind intensity, moisture and activities within the feedlot; i.e. movement of cattle in or out, cleaning, etc., all impact formation of bioaerosols and dust particles containing pathogens.

What does this mean for you?
- Pre-harvest inspection of fields just prior to harvest can be used to identify animal intrusion events and, if found, a harvest buffer zone can be set in place to manage any potential pathogen cross contamination hazards.
- It is important to conduct a comprehensive pre-plant hazard analysis of production locations and to understand the potential for wind-borne contamination from active feed lots prior to planting.
- Where the potential for wind-borne pathogen contamination exists, the use of wind barriers and/or deterrents to dust, e.g. increased moisture may be effective hazard control tools. These factors must be considered in any hazard assessment and the development of management practices.
3. There are no "risky" wild animal species.
A number of studies have been undertaken to examine the potential for animals to harbor human pathogens and transfer them to fruits and vegetables. Field-level experiments and sampling programs have shown that filth flies, several species of birds, reptiles and amphibians, and larger warm-blooded animals like deer, elk, feral pigs and dogs can be carriers of human pathogens like *Salmonella* and *E. coli* O157:H7. Indeed, we have begun to understand that it is not the animal per se, but the potential sources of human pathogen contamination, e.g. concentrated animal feedlots, open sources of raw manure, etc., in the environment in which the animal exists that result in the animal infection and subsequently becoming a transfer vector.

The complex biological interactions between wild animals, the environment and their potential to vector human pathogens to fruit or vegetable crops is an area of intense study. By examining landscape features, land use in adjacent areas, animal movement patterns and prevalence of genetic strains of *E. coli* in soil, animal and water samples, models may be able to be created that might be used to forecast contamination events. While this work is still in the early stages, the prevalence of *E. coli* in environmental samples has been shown to differ between landscapes and different cover types. It also appears that forests that border production fields might be acting as a source of *E. coli* that can be transmitted to the fields since a forest habitat harbors increased genetic diversity and can support higher levels of bacteria than the field environment. This study marks an important departure in industry thinking relative to animal intrusion. It demonstrates how environmental testing data and observation may be used to build predictive models that might assist growers in assessing how pathogens move through production environments and provide insight on how to best manage these potential hazards.

**What does this mean for you?**
- It is important to understand not only the adjacent land use but also the proximal land use to their production fields and the transitory patterns of wild animals in that environment. This is best accomplished through a thorough hazard analysis prior to planting.
- While human pathogens have been found in a diversity of animal species, the frequency is always fairly low; i.e. simply the presence of an animal is not a guarantee of human pathogen cross contamination to the crop. It is prudent to perform pre-harvest hazard evaluations and to use buffer zones to manage potential risks.
- It is important to put data to work. Environmental testing data, e.g. soil, water, animals, etc., may be combined with observational data on animal movements, weather data and crop data to create predictive models that growers might use to more reliably ascertain real cross contamination hazards moving forward.
4. Keep it simple: practical and cost effective preventive controls can be found.

While many questions around food safety in fruits and vegetables involve complex biological interactions, that is not always the case. For example, it was widely publicized that iceberg lettuce harvest knives used to cut the lettuce plant at the base and then remove the core were a significant point of cross contamination. While this assumption was based on the unlikely presence of high concentrations of pathogens in lettuce fields that would be contacted by the knives, there were no commercial data to disprove this theory. Subsequent research has shown that pathogens are infrequently present in commercial fields and when they are, they are present in very low concentrations. Additionally, simple modifications to lettuce coring knives that extend the distance from the cutting knife to the coring ring can significantly reduce the risk of pathogen contamination. Further, by polishing joint welds, the tools are much easier to sanitize thereby further reducing cross contamination frequencies.

Another example where a relatively simple idea might have a significant impact is the use of zero-valent iron to improve irrigation water quality. The microbial quality of many surface or open sources of irrigation water is largely unknown. However, water testing data collected during the execution of a number of funded research programs indicate that open water sources can undergo periodic fluctuations in indicator generic *E. coli* concentrations and in some regions of the U.S., *Salmonella* spp. are routinely found in surface waters used for irrigation. Zero-valent iron (ZVI) holds promise as a water purification system. Preliminary results describing water purification via the use of scrap iron and sand filters might provide a low cost method to remove contaminants from higher risk irrigation water sources, e.g. surface water sources. When iron fragments are stratified and separated by sand layers, water can be passed through this “filter.” It has been shown that *Salmonella* and *E. coli* O157:H7 mixed into the water is bound by the iron, and in some cases, inactivated. While there remains considerable work to be done to translate lab-scale experiments to operationally practical irrigation water purification systems, the technology holds promise as it can be a practical enhancement over sand filters commonly used in production, requires no energy inputs, utilizes scrap iron and represents a renewable method to reduce pathogen contamination in water.
What does this mean for you?
- Equipment surfaces should be designed to eliminate rough edges and cracks that can serve as areas where microorganisms can become established and represent a source of cross contamination to food. Joints should be welded and polished and equipment inspected routinely to insure it is in good repair and easily sanitized. This applies to field, packinghouse and processing equipment.
- Food safety programs should not be rigidly prescribed. Innovation and original thinking are critical tools in addressing food safety questions. Sometimes a relatively simple solution like extending the length of a harvest tool and improving the quality of welds can prove to be an effective preventive control. Low cost, easy to use methods like the use of ZVI to improve irrigation water quality hold the promise of being an effective solution to a worldwide issue.
- It is important that the effectiveness of new preventive controls like ZVI, new equipment designs, etc. are validated under commercial conditions to demonstrate they work as intended and that once incorporated into the operational routine, preventive controls are verified as appropriate.
5. Composting and soil amendment preparation should be viewed as a process with measurable controls.

The use of various composts is a common and necessary practice in the produce industry to improve and restore soil fertility. However, the safe production and application of composts must be viewed as the result of a well-controlled manufacturing process that is monitored and verified. A number of CPS-funded programs have identified key variables that must be understood by compost producers and growers. These include: moisture, heat-up times, time x temperature controls, pH, particle size, C:N ratio, raw material sources, product turns and finished product storage. Even with products like heated chicken pellets where processing temperatures can exceed 300°F, one must be very careful to insure the process is precisely controlled to achieve desired Salmonella population reductions. Failure to properly control any composting or soil amendment preparation process can result in pathogen survival and the development of heat tolerance so that the pathogens are better able to survive and represent a potential cross contamination hazard.

What does this mean for you?

- It is important for growers purchasing composts for use in fields that they know their supplier and that the supplier can demonstrate that the compost was produced according to a validated process and further they can verify that the specific lot(s) being purchased were produced within the parameters of that validated process.
- If a grower is producing composts for use on their farm, they must understand the variables of the composting process and verify that the process they used has effectively reduced human pathogen populations.
- If pathogen or indicator testing is used to verify the efficacy of the composting process, it is important to use sufficient samples sizes and be sure the tests account for the complex organic backgrounds which can affect PCR sensitivity and the presence of non-pathogenic bacterial species.
6. Any wash process must be sufficiently controlled to prevent cross contamination.

Many different products are washed, cooled or transported using water. Therefore it is important that the water is treated and maintained properly so that it does not become a source of cross contamination for human pathogens, should they be present. In other words, understanding your process for water disinfection and validating its efficacy is critical for the safety of the product. It is equally important to remember that simply washing products is not an effective mechanism for removing contamination, i.e. it cannot remove or kill pathogens that have had the opportunity to naturally seek out hidden surfaces on products and adhere to them. CPS-funded research projects have described important variables of your wash water system that must be properly controlled. These include: temperature, pH, turbidity, sanitizer concentration, product load per wash volume, contact time and source water quality. Each type of wash, cooling or product transport system can have different characteristics and physical design so operators must characterize their specific system and validate that their disinfection process or preventive controls are effective and verify that they are operating the system within the validated limits during production runs. Improper control over wash, cooling or water-based transport systems can do harm possibly resulting in large-scale cross contaminations. One CPS-funded program vividly demonstrated this assertion using an inoculated cilantro load and washing it with un-inoculated parsley on a commercial wash system. The improperly controlled wash system permitted cross contamination onto the parsley demonstrating the potential for cross contamination.

More resilient wash water chemistries are emerging. The commercial product, T128 is representative of the “next generation” of chemical wash water sanitation systems. The data generated thus far indicates T128 may act by preserving or “protecting” active chlorine under conditions where increasing organic loads in wash water systems would normally deplete chlorine. In effect, T128 may act as a “safety net” by providing operators protection from cross-contamination. Over time, as organic load builds in wash water using traditional sodium hypochlorite wash water treatment, the amount of active chlorine sanitizer decreases owing to interactions with organic materials. This condition may permit pathogens, if present, to survive in the wash water and cross contaminate the produce as it moves through the system. T128 works by protecting active chlorine as organic loads increase thus diminishing cross contamination risks.
Additionally, reports from CPS-funded scientists revealed that: (1) organic load in tomato wash flumes is a critical factor impacting aqueous chlorine dioxide (ClO2) concentrations, (2) practical steps to reduce vine and leaf trash in flumes would help minimize the rapid rate of antimicrobial oxidizer loss, (3) while oxidation/reduction potential (ORP) is commonly used as an indirect measure of active chlorine in systems where water contacts raw products, positioning of sensors, water temperature and organic load can impact readings, and (4) two-step combinations in some wash systems (e.g. tomatoes) where physical brushing accompanied by a water/sanitizer spray was shown to result in a >3-log reduction in surface microbes.

What does this mean for you?
- Even properly managed wash systems do not sanitize the surface of fruits and vegetables so the multi-hurdle food safety programs that begin pre-plant and extend through the supply chain are needed. Washing is not a kill step. Improperly managed wash, cooling or transport systems using water can be a significant source of cross contamination if pathogens are present.
- Whenever water contacts the surface of fruits or vegetables, it is important that the microbial quality of that water be properly controlled and monitored. Operational parameters should be developed for the system and the performance of preventive controls should be validated and then verified during use.
- New strategies for washing produce are emerging. Combinations of treatments may offer better and more efficacious control over microorganisms in wash water. Operators needs to monitor and evaluate emerging technologies and test them relative to their unique process requirements.
7. Breeding fruit and vegetable varieties for resistance to human pathogens?

Plant genetics and physiology may play a role in pathogen survival. Two research programs hinted at the role plant genetics may play in pathogen contamination and survival on spinach leaves and tomato fruits. Historically the industry and the research community have focused on a better understanding of the growing environment, pathogen vectors and the genetic and physiological attributes of the pathogen. The data presented on *Salmonella* survival on a broad collection of tomato varieties and *E. coli* interactions with slow and fast-growing spinach leaves may indicate that the genetic and physiological state of the plant may also impact survival of pathogens on plant surfaces. It is unclear whether it may be possible to select for genetic resistance to human pathogens in the future, but a better understanding of plant/human pathogen interactions will help inform future research and risk management strategies.

What does this mean for you?

- Stakeholders in the produce supply chain need to monitor food safety research to have a view to emerging trends and concepts. This base of knowledge will ultimately permit more effective communications with the scientific and seed breeding communities to insure that priority is given to the most promising avenues of research. Certainly unlocking the genetic potential of the plant in combating human pathogen survival is an important avenue to explore in improving food safety.
8. Seek and destroy is a strategy for managing potential *Listeria monocytogenes* hazards.

The 2013 CPS Symposium included a workshop focused on Listeria biology and lessons learned on *L. monocytogenes* control from the meat and produce industries. There are many basic differences between *L. monocytogenes* and other human pathogens like *Salmonella* and *E. coli* O157:H7. One of them is that *L. monocytogenes* can become a resident and persistent problem if it is permitted to establish itself in a produce processing or packing operation. The processed deli meat industry faced similar issues with *L. monocytogenes* ten years ago and adopted a "seek and destroy" strategy, i.e. a robust search for niches where Listeria might become established and implementation of aggressive sanitation programs to prevent Listeria from "moving in" and becoming resident. Key elements of a preventative program are: comprehensive Good Agricultural Practices (GAP) program at the field level to keep the incidence of Listeria introduction into the packing or processing environment low, work flow patterns within the facility that reduce cross-contamination potential, equipment design that reduces potential Listeria harborage areas and facilitates thorough cleaning and sanitation and a risk-based environmental testing program.

**What does this mean for you?**

- It is critical that operators develop a comprehensive plan for environmental testing and be committed to conducting root cause analysis when positive results are obtained to ensure that the reasons for the positive tests are understood and corrective measures put in place to prevent a reoccurrence.
- Facility and equipment design needs to reflect the potential for *L. monocytogenes* contamination and to permit effective cleaning and sanitation.
- Operators need to have written facility and equipment sanitation programs with verification measures to insure consistent performance.
9. *Salmonella* species can adapt to production environments.
A re-current theme across several research projects has been the hardiness of *Salmonella* in the soil, on soil amendments and on plant tissues. Research on *Salmonella* survival on chicken pellet soil amendments demonstrated that manufacture and production of finished chicken pellets has to be thought of as a manufacturing process with critical measures like moisture level and temperature fastidiously controlled to insure the pathogen is eliminated otherwise it can build a resistance to heat treatments. Similarly, attenuated *Salmonella* was shown to survive much better than attenuated *E. coli* O157:H7 when inoculated onto spinach or romaine leaves, chopped and disked into the soil in the Salinas Valley. Another project demonstrated that *Salmonella* serovars were much more resistant to the antimicrobial effects of natural isothiocyanates from broccoli versus *E. coli* O157:H7 strains. Lastly, data has been presented that indicated that if *Salmonella* becomes desiccated, its ability to survive can be increased. If *Salmonella* are grown in liquid broth they are much less hardy than *Salmonella* grown on agar plates. This observation has important ramifications for researchers and the design of experiments testing survivability of *Salmonella* in produce environments such as pack houses and fields. It also reminds produce industry operators that the environment in which produce is handled or processed (wet to dry transitions, rapid temperature changes, sanitizer concentrations, etc.) can affect the survivability of *Salmonella* and the potential for cross contamination hazards.

**What does this mean for you?**
- All operators within the produce supply chain need to consider the potential for *Salmonella* contamination at all levels via a comprehensive hazard analysis and establish preventive controls that effectively diminish the potential hazard.
- Preventive controls need to be validated and their execution must be verified and carried to completion to prevent potential *Salmonella* contaminates from developing tolerances to the treatments.
10. Irrigation water and understanding public health risks.

Large segments of the produce industry currently perform some type of irrigation water testing. Generally, generic *E. coli* is used as an indicator for fecal contamination in irrigation water tests. Detection of generic *E. coli* is not necessarily an indicator for pathogenic *E. coli* strains or *Salmonella* spp. However, data collected over time can be used to establish baseline performance for a water source and any significant deviations from that baseline can be an indicator that the source and/or the delivery system has been compromised and the grower should perform an inspection of the system. One CPS-funded study used over 60,000 California industry irrigation water test results to demonstrate the very low incidence of generic *E. coli* concentrations above the EPA recreational water standard (256 MPN/100 mls for a single test, 126 MPN/100 mls on a five test rolling mean). There were clearly differences in exceedances when comparing closed water sources like deep wells and open water sources such as on-farm ponds and canals. Each irrigation water source should be evaluated for potential contamination risk factors that must be evaluated and managed; e.g. risk of animal intrusion, potential for run-off, water delivery mechanisms, seasonality, etc. A comparative study focused on generic *E. coli* test methodologies pointed out that growers need to be sure that the tests used to measure generic *E. coli* have the proper sensitivity. While several tests are available, it is important that the detection level of the kit is matched to the standard. For example, if the target is the EPA recreational water standard of less than 126 MPN *E. coli*/100 mls, then a test sensitivity of 200 MPN *E. coli*/100 mls would be inappropriate. Data have also been developed that demonstrate the value of using larger water samples to enhance the probability of detecting low levels of contamination.

In one of the first examples where quantitative microbial risk assessment (QMRA) has been applied to produce food safety, irrigation water test data and a series of assumptions around time intervals from final irrigation to product consumption, serving sizes and irrigation practices, relative public health risks were calculated. For example, data has been presented that shows sub-surface drip irrigation with water containing 126 MPN generic *E. coli*/100 mls could result in 9 illnesses/100,000,000 consumers compared to 1.1 illnesses/1,000,000 consumers if furrow irrigation were used and 1.1 illnesses/1,000 consumers if sprinkler irrigation were used. The data clearly show that public health risk is a function of source water quality and irrigation delivery system used. As in all models, the model is only as useful as the quality of the data and the assumptions made to build it. However, the QMRA model is very useful in helping growers prioritize and manage potential contamination risks.
What does this mean for you?

- Growers, harvesters and processors should include irrigation water sources and delivery systems in any hazard assessment conducted for their operations. Potential sources of contamination should be identified and preventive controls developed to manage hazards.
- Irrigation water testing can be a useful tool to establish baseline performance of irrigation water systems. An irrigation water testing data base may permit growers to identify seasonal hazards and facilitate risk-based testing programs that are more cost effective and more effective in managing contamination hazards.
- Operators that perform irrigation water testing should have written protocols for taking samples and a rationale for the test method used, sample size and what actions are to be taken should the results exceed operating parameters.
- It is time to put our data to work. QMRA represents a useful tool to help the industry quantitate public health risk related to various agricultural inputs or processes. Ultimately this will enable the industry to prioritize risks and expend resources against those areas that can most effectively improve safety.
11. Testing is about sampling strategies.

Product, environmental and water testing have become part of the food safety landscape in the produce industry. A recurrent result among CPS-funded research programs has been that larger sample sizes increase the chance of finding pathogens. Typical commercial product sampling procedures use 25-gram samples of plant tissues and 100-ml samples for water to test for pathogens and/or indicator organisms. Data from a number of projects have shown that increasing the sample size to 150-grams for products and >200 mls for water increases the chance of detecting low level contaminations. Sporadic contamination frequencies and the low concentration of contaminates, make sampling strategies difficult to develop. This reality has been characterized as “finding a needle in a haystack”. One research project provided a stark example of this reality. Raw almonds were collected by handlers over a period of a decade. Of the nearly 15,000 samples collected and tested for *Salmonella*, the frequency of contamination was generally between 1-2 percent and the concentration of *Salmonella* was <1 MPN/100 grams. Over time, the samples that tested negative were held in storage. At the end of the study, the researchers went back and sub-divided the “negative” samples and retested them for *Salmonella*. Approximately 1 percent of the samples were found to be “positive” for *Salmonella*. These results show the limitations of sampling when the frequency and concentration of pathogen contamination are so low. “Positives” can be shown with confidence to be positive, but “negative” samples may not necessarily be negative.

**What does this mean for you?**

- It is important to consider the benefits and deficiencies when determining the role of testing to verify food safety programs. If testing is to be used, there should be a written program describing the objectives of the program, the sampling strategy to be employed, the microorganisms to be tested and the protocol to be followed in executing the test, the sensitivity and selectivity of the protocol and how the data will be evaluated and stored.
- When testing is employed, efforts should be taken to use as large a sample as is practical.
- Any operation that employs product, water or environmental testing should develop plans for what actions need to be taken when the test results are “negative” and when they are “positive”. Negative test results generally mean it is acceptable to use that water or product or that the sanitation program was effective. Positive test results can elicit a number of different actions and it is important to plan ahead and have a plan for how the organization should react.
12. Clean and sanitize surfaces that come in contact with products.

Produce handling results in contact between the product and various surfaces that can become contaminated with pathogens. There have been CPS-funded research programs dealing with the potential for transference of pathogens from contaminated gloves, cloths used for wiping fruits, product cartons and plastic harvest buckets. The use of gloves has been debated within the produce industry for several years. Data suggests that hand wash prior to use of any kind of glove is very important and that the gloves need to be sanitized as they are used. Nitrile gloves do not facilitate cross contamination as well as latex gloves, however both types will transfer pathogens if not cleaned and sanitized regularly with a sanitizer. Another frequent point of contention in the industry is the potential for pathogen transference owing to the use of cloths to wipe fruit that is field packed. Again, data have been developed that shows that pathogens can be transferred from fresh tomatoes to cloths and from cloths to subsequently handled tomatoes. While there are many factors at play, moist cloths facilitate transference more readily than dry cloths and dirty cloths seemingly are less efficient at transference than cleaner cloths, although both can facilitate transference if pathogens are present.

The question of pathogen transference from cartons or harvest buckets has also been addressed. Specifically, the re-use of cartons has been examined and it has been demonstrated that cartons that have organic residues and moisture from their initial use can indeed transfer pathogens to fruit re-packed in those cartons. Additionally, pathogen transference has been studied in multiple-use containers like tomato harvest buckets. Variables such as the age and condition of tomato harvest buckets were considered when determining their potential as pathogen transference vehicles. Surprisingly, older, scratched and worn plastic buckets were less effective in transferring inoculated Salmonella than newer buckets. Once again, new and old buckets could affect transference if the pathogen was present and the presence of soil on the buckets decreased Salmonella die-off. This emphasizes the importance of regularly cleaning and sanitizing harvest containers to prevent transference of pathogens to harvested products.
What does this mean for you?

- If gloves are used to handle raw products, they should be changed frequently and/or cleaned and sanitized periodically while in use. Preference should be given to the use of nitrile gloves. Hand washing should always be a prerequisite to using gloves.
- The use of cloths to clean or wipe dirt off harvested tomatoes should be avoided. While the contamination frequency would be expected to be very low on the surface of tomato fruits, if a pathogen were there, the cloth could spread that contamination across several fruits.
- It is important to store produce cartons so that they remain dry and clean. If cartons are to be re-used, e.g. re-pack operations, they should be thoroughly inspected and cartons that are wet or have dirt or debris in them should be avoided.
- Re-usable containers like harvest buckets should be periodically cleaned and sanitized. Containers should be visually inspected on a routine basis to be sure dirt and other field debris does not accumulate.

This paper was developed based on data shared by participating scientists at the Center for Produce Safety Research Symposia from 2010 to 2013. The statements made here are interpretations and recommendations.

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