UNITED STATES DEPARTMENT OF AGRICULTURE
SMALL BUSINESS INNOVATION RESEARCH
SOLICITATION NO. USDA / 02-1

OMB Approved 0524-0025

PHASE I AND PHASE II
PROPOSAL COVER SHEET

Proposal No. (for USDA use only)
Date Received

SUBMITTED	**Firm:** Lynntech, Inc.
	Mailing Address: 7610 Eastmark Drive, Suite 202 College Station, TX 77840

Project Title: A New Technique for Ante-Mortem Control of Pathogens in Broilers

Topic No. and Area (check appropriate box; see Section 8.0)

9 8.1 Forests and Related Resources	9 8.4 Air, Water, and Soils	9 8.7 Aquaculture
9 8.2 Plant Production and Protection	9 8.5 Food Science and Nutrition	9 8.8 Industrial Applications
8 8.3 Animal Production and Protection	9 8.6 Rural and Community Development	9 8.9 Marketing and Trade

Amount Requested: ($) $65,000	**Proposed Duration (Mos.):** 6	**Congressional District No.:** 8th	**YES**	**NO**
1. The above concern certifies that it meets the first two criteria of a small business concern as stated in this solicitation (See subsection 2.2).			✘	
2. The above concern certifies that it qualifies as a socially and economically disadvantaged small business as defined in this solicitation (See subsection 2.4). (For statistical purposes only).				✘
3. The above concern certifies that it qualifies as a women-owned small business as defined in this solicitation (See subsection 2.5). (For statistical purposes only).				✘
4. The above concern certifies that the Principal Investigator's primary employment (at least 51%) will be with proposing firm at the time of any resulting award and during the conduct of the proposed research (See subsection 2.2(C)).			✘	
5. The above concern certifies a minimum of two-thirds of the research (phase I) or one-half the research (phase II) will be performed by this firm (See subsection 2.2(D)).			✘	
6. Will you permit the Government to disclose the title and technical abstract page of your proposed project, plus the name, address, and telephone number of the corporate official of your firm, if your proposal does not result in an award, to entities that may be interested in contacting you for future information?			✘	
7. Do you plan to send, or have you sent, this proposal or a similar one to any other Federal agency? If yes, give acronym(s); e.g., DOE, NIH, NSF, etc.				✘
8. Is the organization delinquent on any Federal Debt? (See subsection 5.11). (If yes, attach explanatory information).				✘
9. Will the work in this proposal involve recombinant DNA, living vertebrate animals, or human subjects? (If yes, complete Form CSREES-2008).			✘	
10. Is this proposal a resubmission of a proposal submitted earlier to the USDA SBIR Program (See subsection 3.3(D)). If yes, list the proposal number _____.				✘

By signing and submitting this proposal, the prospective grantee is providing the required certifications set forth in 7 CFR Part 3017, as amended, regarding Debarment and Suspension and Drug-Free Workplace; and 7 CFR Part 3018 regarding Lobbying. (Please read the Certifications and Instructions included in this solicitation before signing this form.) In addition, the prospective grantee certifies that the information contained herein is true and complete to the best of its knowledge and accepts as to any grant award, the obligation to comply with the terms and conditions of the Cooperative State Research, Education, and Extension Service in effect at the time of the award. *Submission of the Social Security Number is voluntary and will not affect the organization's eligibility for an award. However, it is an integral part of the CSREES information system and will assist in the processing of the proposal.

PRINCIPAL INVESTIGATOR		**AUTHORIZED ORGANIZATIONAL OFFICIAL**	
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PROPRIETARY NOTICE (IF APPLICABLE, SEE SUBSECTION 5.4)

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U.S. DEPARTMENT OF AGRICULTURE

SMALL BUSINESS INNOVATION RESEARCH

PHASE I AND PHASE II

PROJECT SUMMARY*

OMB Approved 0524-0025

FOR USDA USE ONLY			
Program Office	Solicitation No.	Proposal No.	Topic. No

TO BE COMPLETED BY PROPOSER

Name and Address of Firm	Name and Title of Principal Investigator(s)
Lynntech, Inc. 7610 Eastmark Drive, Suite 202 College Station, Texas 77840	G. Duncan Hitchens Vice President/Senior Research Scientist

Title of Project (80-character maximum)

A New Technique for *Ante-Mortem* Control of Pathogens in Broilers

Technical Abstract (200-word limit)

Contamination of poultry and poultry products by *Salmonella* and other pathogens is a serious world-wide problem. One study has shown 1.4 billion dollars in lost productivity, medical expenses, and increased annual production costs in the U.S. caused by *Salmonella* alone. For this reason, methods to control *Salmonella* and other food-borne pathogens on poultry are a research priority. A contributing factor to poultry carcass contamination is the presence of human pathogens throughout the animals' gastrointestinal tract at the time of slaughter. Therefore, measures to reduce pathogens are needed during the pre-slaughter period. This proposal describes a method for intervening in the contamination of broilers by providing drinking water containing a potent disinfectant. The supplemented drinking water will minimize colonization of upper gastrointestinal tract of the chickens, which is an important source of pathogens like *Salmonella*. The disinfectant solution is safe to use on foods and will leave no chemical or environmental residue. A low-cost, miniature device will generate and self-administer the disinfectant to the drinking water without a significant modification to the broiler facility and minimum intervention by the grower. The method is complementary to and easy to integrate with other *ante-mortem* pathogen reduction programs. The Phase I will investigate the feasibility of the method in collaboration with researchers at the Poultry Science Research Center at Texas A&M University.

Anticipated Results/Potential Commercial Applications of Research (100-word limit)

Salmonella contamination of broiler products is a continual problem for the poultry industry. The technology described in this proposal will fill a gap in current broiler management practices and has potential to significantly reduce the incidence of pathogens from final store-ready products. The improved quality of the product will ultimately be passed on to the consumer which can only benefit the poultry industry.

Keywords to Identify Technology/Research Thrust/Commercial Application (8-word maximum)

Food Safety, Broiler Carcasses, *Salmonella*, Pre-Slaughter, Water Disinfection, Feed Withdrawal

*The Project Summary must be suitable for publication by USDA in the event of an award. Do not include proprietary information on this page.

Form CSREES-668 (7/87)

C1. IDENTIFICATION AND SIGNIFICANCE OF THE OPPORTUNITY

Salmonella contamination continues to be a potential problem for the broiler industry. Improvements in processing procedures and sanitary methods within processing plants have allowed for general microbiological improvements in overall carcass quality through the initial stages of processing. However, the incidence of *Salmonella* on broiler carcasses has been shown to increase with successive stages of processing (Lillard, 1989), possibly due to *Salmonella's* ability to firmly attach to poultry tissue. Much research has focused on cecal and intestinal content contamination (Fanelli *et al*., 1971; Corrier *et al.*, 1990) as the primary source of *Salmonella* within chickens. However, recent reports have shown the crop may potentially serve as an important source of *Salmonella* contamination on broiler carcasses within some processing plants (Hargis *et al*., 1995). A higher incidence of *Salmonella* in crops than in ceca has been reported, along with a higher incidence of ruptured crops than ruptured ceca during commercial evisceration. In addition, colonization of the crop by *Salmonella* can increase as chickens near processing age (Humphrey *et al.*, 1993; Ramirez, *et al.*, 1997). Consequently, the crop is now considered an important critical control point for reducing *Salmonella* contamination of broiler carcasses.

Our goal is to develop a method for intervening in the contamination of the crop as broilers reach marketable age. We will demonstrate a new bird watering method that provides broilers with oral antiseptic solutions containing dissolved ozone. The concept is shown in Figure 1. The aim is to provide a drinking solution that minimizes bacterial colonization of the crop and upper gastro intestinal tract of the chickens at the critical pre-slaughter time (See Figure 2). Recently, ozone solutions have been studied as an antiseptic for intestinal disorders in humans. This research has shown that ozone solutions are safe when taken internally and that they offer a high potential for minimizing bacterial colonization of the digestive system. The benefits of ozone include its high solubility in water (ten times that of oxygen) and a strong capability to eliminate many different kinds of microorganisms. Yet ozone does not persist, it rapidly decomposes into oxygen leaving no harmful residues. In 1997, ozone was conferred GRAS (Generally Recognized as Safe) status for use as a disinfectant on foods by the Food and Drug Administration (Majchrowicz, 1998; Federal Register, 1997; Graham, 1997; Anon, 1997). Ozone has been used safely and effectively to purify drinking water for nine decades. It also has GRAS status for use in bottled water.

We will use a unique miniature ozone generation-injection device that connects directly into existing bird waterers. The method has been devised to be minimally intrusive, so that the operator can temporally attach the ozone generator onto water lines close to the point of consumption through a quick-connect fitting. The device is designed for continuous operation during the time of feed withdrawal, leading up to crating and transportation. The device can be quickly removed and transferred to other rearing areas as required. The projected cost of the miniature ozone generator-injector is $50-100. The ozonation hardware we will use is based on existing designs (Hitchens, *et al.*, 1994; Murphy, *et al.*, 1994; Murphy & Hitchens, 1995; Anon, 1997b; Murphy & Hitchens, 1998); therefore, the proposed equipment build-up needed for all aspects of this project will be accomplished in a timely manner with little or no requirement for ozone technology development.

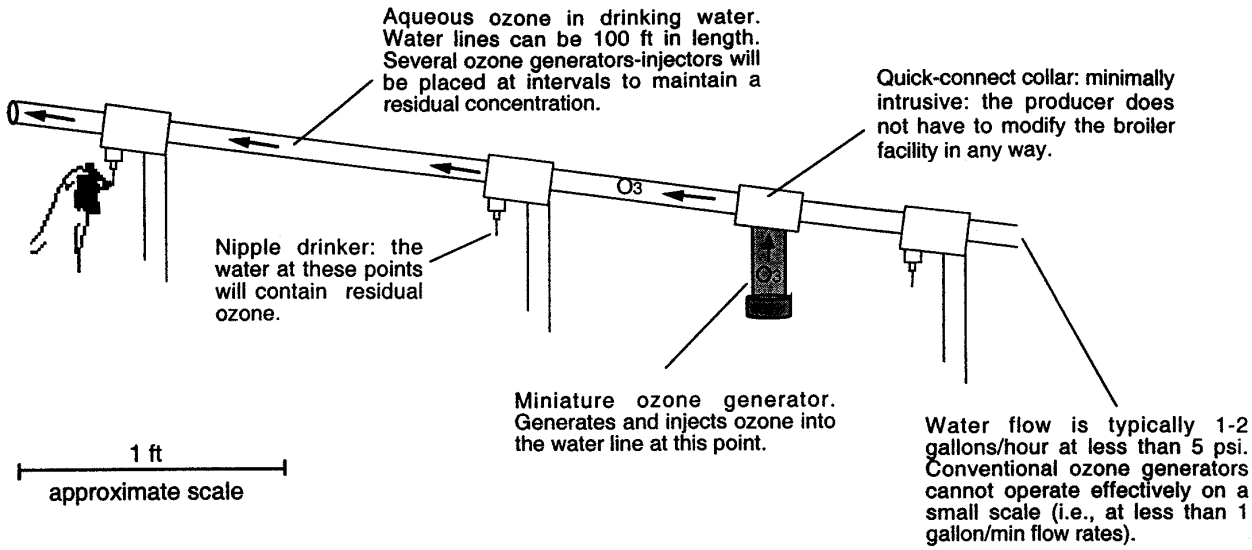

Figure 1. The concept for providing dissolved ozone for broiler drinking water. Ozone is generated in a unique electrolysis process. The generators also inject ozone into the water line without interfering with the normal operation of the waterer. Ozone can be effective against *Salmonella*, *Campylobacter,* viruses and other emerging pathogens and offers the potential for decontamination of ingesta in the crop and other regions of the GI tract. Self-disinfection of the water lines and watering equipment is also provided and ozone can be applied directly to incoming municipal water supply or well water; ozone is non-reactive with chlorine. The miniature ozone generators are expected to cost $50-100 per unit.

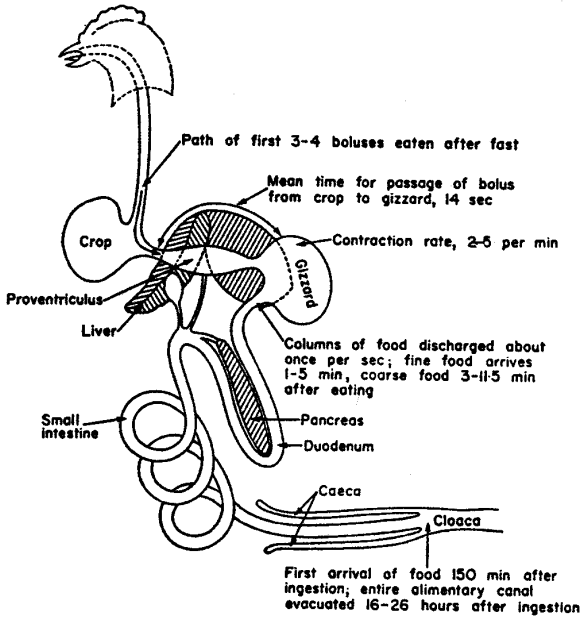

Figure 2. The Phase I goal is to demonstrate that ozonated water can be ingested by broilers to reduce *Salmonella* colonization of the crop. Ozone solutions are used as a therapeutic agent for intestinal disorders in humans and will not leave harmful residues in the bird or in water. The presence of dissolved ozone at mg/L concentrations is not easily discernible in drinking water. Therefore, the ozonation method should not affect the palatability of the drinking water. Diagram adapted from Malden *et al.*, 1979.

During this project, a subcontract will be made to Dr. Billy M. Hargis, Professor, Department of Veterinary Pathobiology, College of Vet. Medicine, Texas A&M University. Dr. Hargis is director of a leading research laboratory in poultry diseases and has studied *Salmonella* contamination of the crop. The blend of technical competencies between Dr. Hargis and Lynntech, Inc. provides a very effective team with strengths in oxidative disinfection coupled with a thorough understanding of microbial diseases. The background section that follows describes relevant literature on broiler carcass contamination by *Salmonella* and other pathogens. The section also discusses the current status of ozone in the food industry, as well as research on ozone solutions for internal treatments in humans.

C2. BACKGROUND AND RATIONALE

BACKGROUND

(I). Contamination of Broiler Carcasses

The prevalence of *Salmonella* and *Campylobacter* on retail poultry carcasses remains a significant public health concern. The Public Health Service/Centers for Disease Control report that each year millions of Americans suffer illness caused by foodborne infection. *Salmonella* and *Campylobacter* together are thought to be responsible for the majority of acute cases of gastroenteritis (Mulder, 1995). The global association between the occurrence of these genera of foodborne pathogens and contamination of poultry are well documented in the literature (Lahellec & Collin, 1985; Marinescu *et al.*, 1987; Lammerding *et al.*, 1988). In an attempt to characterize the *ante-mortem* levels of pathogens in commercial broilers, Jacobs-Reisma and coworkers (1994) found that, of over 180 flocks surveyed approximately, 27% contained *Salmonella* and 82% contained *Campylobacter*. More recently, Ramirez and coworkers found from 19-36% of commercial broilers (n=100) contained *Salmonella* in the crops and ceca just prior to slaughter (1997). A 1983 survey of poultry carcasses showed that of 215 carcasses that exited the chiller bath at the slaughter facility, 11.6% were positive for *Salmonella* (Campbell *et al.*, 1983). Stern and Line (1992) found *Campylobacter spp*. by extensive analysis in 98% of retail packaged broilers. The correlation of infected birds to contamination of the final product seems to, therefore, be a linear relationship, warranting intervention strategies at *ante-mortem* stages of production.

Much of the research regarding the source of pathogen contamination of poultry has focused on cecal and intestinal content contamination (Corrier *et al.*, 1990), with the presumed major reservoir of pathogens being expelled onto the carcass via emptying of the cecal contents during processing (Fanalli *et al.*, 1971, Snoeyenbos *et al.*, 1982). However, recent reports have identified the crop as a significant harbor of pathogenic bacteria and therefore, this upper G.I. organ may be serving as an additional source of contamination on broiler carcasses (Hargis *et al.*, 1995; Ramirez *et al.*, 1997). Supporting evidence for this hypothesis may be found in a study by Hargis and coworkers who found that the incidence of crop rupture in commercial evisceration is higher than cecal rupture (1993).

Of additional concern to the broiler industry is the increase in recoverable *Salmonella* in the crops of broiler chickens as the feed withdrawal time period is increased prior to shipment of the birds to the slaughter facility (Humphry *et al.*, 1993; Ramirez *et al.*, 1997). These data further suggest that *ante-mortem* management practices may influence the degree of carcass contamination at slaughter. Indeed, this was the

approach used by the developers of competitive exclusion (CE) innoculums for chicks (i.e., Preempt, which was developed by USDA scientists and MS Bioscience), which utilizes indigenous gastrointestinal microflora to compete for resources and therefore, exclude the proliferation of more harmful, pathogenic bacteria (Byrd *et al.*, 1998). It is clear that the period of feed withdrawal is coupled with consumption of the litter, a harbor of *Salmonella* that contaminates both the ceca and crop (see Table 2).

Table 2. Effect of Feed Withdrawal on *Salmonella* Colonization of the Crop and Ceca in Market Age Broiler Chickens (Adapted From Ramirez, *et al.*, 1997).

Expmt.	Treatment*	Positive crops/ total	Positive ceca/ total
1	FF	4/14 (29%)	9/15 (60%)
	WF	12/15 (80%)	14/15 (93%)
2	FF	3/25 (12%)	11/25 (44%)
	WF	22/25 (88%)	11/25 (44%)
3	FF	3/20 (15%)	7/20 (35%)
	WF	16/20 (80%)	15/20 (75%)
4	FF	5/20 (25%)	14/20 (70%)
	WF	16/20 (80%)	20/20 (100%)
5	FF	19/100 (19%)	25/100 (25%)
	WF	36/100 (36%)	31/100 (31%)

*FF = full-fed, WF = feed withdrawal (18 h withdrawal in Experiments 1 to 4, 8 h withdrawal in Experiment 5). Broilers were orally challenged with 1 x 10^8 *Salmonella entertidis* at 6 wk of age and samples were collected at 7 wks of age (Experiments 1-4). Naturally occurring *Salmonella* were cultured from a commercial broiler house at 7 wk of age in Experiment 5.

The CE approach is excellent for continuous control of *Salmonella* infection of birds throughout the growing period for broiler chicks. However, the most effective location for CE microbes is in the lower GI tract, including the cecum and intestines. By adding an orally administered biocide/biostat through the drinking water during feed withdrawal, the levels of litter-derived *Salmonella* and *Campylobacter* can also be controlled in the upper GI region (Barnhart *et al.*, 1998a, 1998b), thus allowing for the two technologies to work together. The drinking water oxidant proposed in this study will not leave any residue in the bird, its urine or litter, making it an inexpensive, safe, environmentally and consumer friendly alternative to organic acids, salts and antibiotics. The short half life of aqueous ozone and reactivity will mean that ozone and competitive exclusion will work in tandem, at both anatomical locations responsible for harboring pathogens.

(II). Ozone as a Disinfectant

Dissolved ozone is a highly efficient disinfectant-sterilant for all classes of microorganisms (Rose *et. al.*, 1994; Foller, 1982, Takahashi &Nakai, 1994; Zhouu & Smith, 1994; Shen & Ku, 1995; Andreozzi *et. al.*, 1995; Langlais, 1991). The effectiveness of ozone gas as a disinfectant is shown in Table 3. The Table shows ozone to be a non-selective agent for a wide range of bacteria, spores, and viruses. Over the last 100 years ozone has been used in Europe as a disinfectant for water. Ozonation, unlike other chemical treatments, leaves no residual chemicals in the water stream i.e., ozone is a non-persistent chemical. After it reacts, it breaks down to form oxygen gas.

Table 3. Disinfection Features Of Ozone (Nebel & Nezgod, 1984)

Organisms	$C_{t99:10}$
Escherichia coli	0.001
Streptococcus faecalis	0.0015
Mycobacterium tuberculosis	0.05
Polio virus	0.01
Bacillus megaterium (spores)	0.1
Entamoeba histolytica	0.03

$C_{t99:10}$ = Residual ozone concetration in mg/L for 99% destruction in 10 minutes

Temperature = 10 - 15 ÞC **pH** = 7.0

(III). The Use of Ozone in the Food Industry

In recent years, there has been a drift away from conventional chlorine-based water treatments and aqueous ozone technology is beginning to emerge as an attractive alternative. One field in which ozone technology is coming to the fore is in the food industry. Ozone was recently given the status Generally Recognized As Safe (GRAS) by the Food and Drug Administration for use in the food industry. This was accomplished after an expert panel, assembled by the Electric Power Research Institute (EPRI), concluded ozone is safe and a necessity as a sterilant in the food industry (Anon., 1997). The streamlined approach to granting of GRAS status was announced by the FDA in 1997 (FDA, 1997).

Ozone has also been demonstrated to be effective in reducing microbial counts in several areas: increase storage life of meat, fruit and cheeses (Easton, 1951), and to control post-harvest decay of table grapes (Sarig *et al.*, 1996). Ozone is also more effective at disinfecting *Salmonella, Giardia, E. coli* and *Cryptosporidium* than existing chlorine-based technologies (Agricultural Technology Alliance, 1998). Ozone is also capable of degrading a wide range of organics, including pesticide residues (Food Industry Currents, 1997). Ozone has been demonstrated to be an effective food germicide and can significantly reduce the numbers of pathogens on poultry (Dickson, *et al.*, 1992; Yang and Chen, 1979a, Yang and Chen, 1979b). The use of aqueous ozone has been shown to be effective at eliminating both gram negative and gram positive microflora from the surface of poultry meat.

(IV). The Use of Ozonated Water in Eliminating Oral and GI Tract Pathogens

Ozone is approximately 10 times more soluble in water that oxygen. Ozonated water is a common item found in European dental surgeries. In a comprehensive study (Turk, 1985; Filippi, 1997) it was found that ozonated water, when administered orally, promoted hemostasis, enhanced local oxygen supply, and inhibited bacterial proliferation. Ozonated water has also been used as a oral rinse during and after tooth extraction (Sunnen, 1987). Ozonated water has also been used in the treatment of oral cavity infections such as thrush, periodontal disease, and tonsillitis (Silva & Wong, 1998).

Peroral ingestion of ozonated water has also been shown to be effective at treating gastro intestinal problems. Problems such as gastritus or gastric carcinoma have been successfully treated with ozonated water. Androsov *et al.* showed that ozonated water was effective at destroying *Heliobactor pylori* in the patients stomach without causing any side effects. Peroral ingestion of ozonated water has also been used in the treatment of chronic intestinal or bladder inflammation. Ozonated water bubbled into warm baths has been shown to provide stimulation of the local circulation and disinfection action to varicosities, peripheral circulatory disorders, and dermatological conditions (Rilling & Viebahn, 1987). In most of these cases, the ozonated water is prepared using a medical ozone generator which uses pure oxygen instead of air as the gas feed. DI water was borbotaged by the ozone oxygen mixture for 10 minutes then immediately administered to the patient in 100 mL portions.

Figure 3. Photograph of a 20 mg/hr electrochemical O3 generator. Our knowledge of the engineering, materials, and safety aspects of O3 systems is extensive. The ozonation hardware we proposed will be based on existing designs; therefore, much of the proposed equipment build up described in Task 1 of this proposal will be accomplished in a timely manner with little or no requirement for ozone technology development. The electrochemical unit shown in this photograph can be readily adapted into "nipple" –or- "bell" type waterers. Operation aspects of this unit are depicted in Figures 4 and 5.

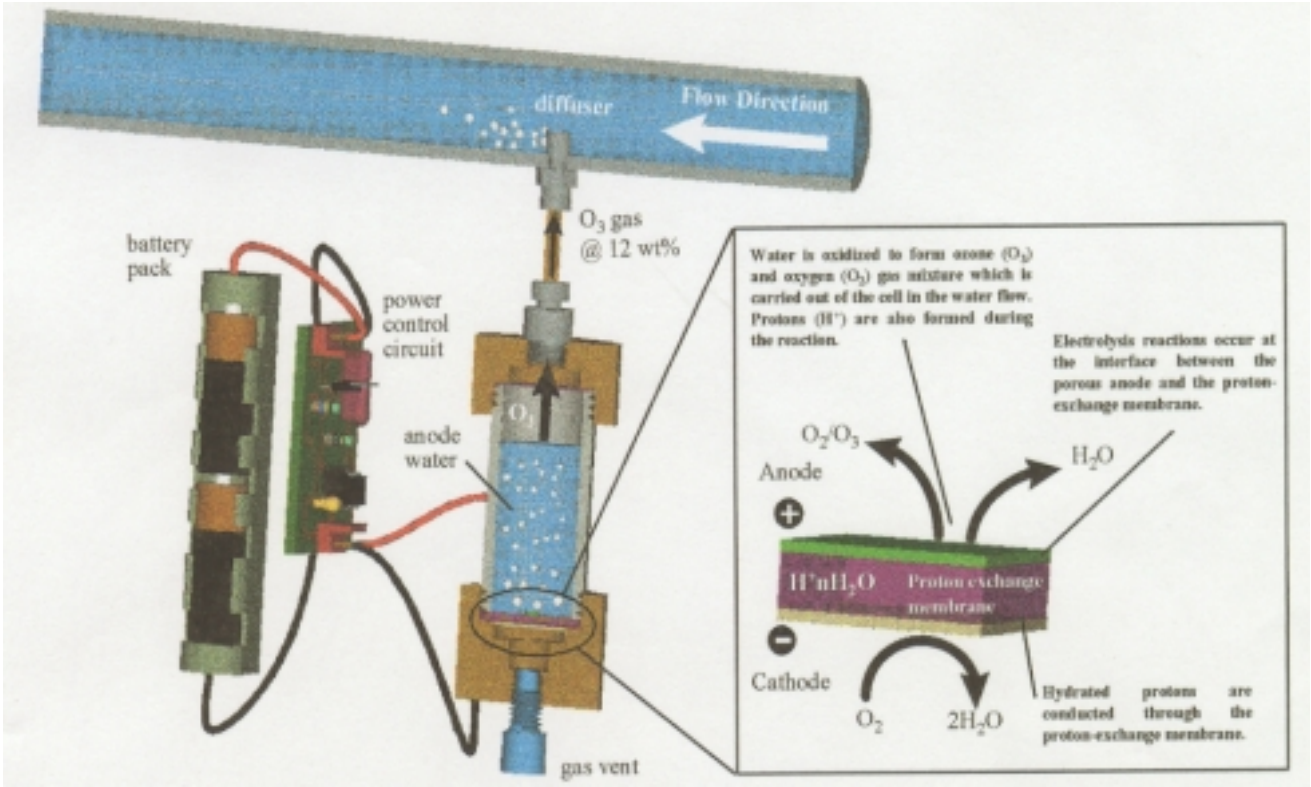

Figure 4. Component layout diagram of the electrolysis apparatus in Figure 3. To minimize equipment costs, *operation does not require valves or pumps*. The expected cost to manufacture is less than $100 each. The unit is entirely self-contained with its own power supply, water management, and waste gas handling systems.

Figure 5. Principle of electrochemical ozone generation in a proton exchange membrane.

RATIONALE

This section describes the new device for ozone generation that will be used for providing ozonated drinking water for broilers. The method uses a unique electrolysis (i.e., electrochemical) process that has been pioneered by Lynntech, Inc., (Hitchens, *et al.*, 1994; Murphy, *et al.*, 1994; Murphy & Hitchens, 1995; Murphy & Hitchens 1998) and is currently being commercialized (Anon, 1997(b)). A photograph of one of our devices is shown in Figure 3. Figure 4 gives the layout of the hardware. Sources of electrical power and water are the only requirements for producing ozone by this method. This method has many unique cost and process advantages for use in small sized water lines. As discussed later, conventional ozone generators (either corona discharge or UV lamps) do not scale down and are impractical for low flow rate water treatment regimes (i.e., for treating 500 L/hr or less).

(I). Principle

Figure 5 depicts the principle of Lynntech's electrochemical ozone generation process. In the process, water is electrolyzed at the anode (a metal oxide electrode), to form a mixture of O_2 (equation 1), and O_3 (equation 2).

$$2H_2O - 4e^- \rightarrow O_2 + 4H^+ \qquad E° = 1.23V \qquad (1)$$
$$3H_2O - 6e^- \; O_3 + 6H^+ \qquad E° = 1.51\ V \qquad (2)$$

The current we apply is typically 1.5-2.0 A/cm^2 of electrode area. The cell voltage is 3.5 V. Approximately 15% by weight of the resulting gas is ozone. The remainder is oxygen. The O_3 and O_2 partition between the liquid and gas phases as they are formed. Protons formed at the anode are conducted to the cathode through a Nafion proton exchange membrane which serves as a solid polymer electrolyte (i.e., the proton conducting pathway between the two electrodes). The use of a Nafion membrane eliminates the need for a liquid electrolyte and acts as a separator between the anode and cathode compartments. Nafion is a fluoropolymer and displays a very high resistance to chemical attack by ozone. The preferred cathodic reaction is the reduction of oxygen, where air serves as the oxygen source. This reaction is represented by equation (3).

$$O_2 + 4H^+ + 4e- \; 2H_2O \qquad E°=1.23\ V \qquad (3)$$

Specialized gas diffusion electrodes are required for the oxygen reduction reaction to occur efficiently. The layer of bonded carbon particles serves as a three-dimensional microporous structure for diffusion of the reactant gas (air) into the electrode structure.

(II). Performance Characteristics

This electrochemical ozone generator is ideal for small capacity drinking water applications. Some of its characteristics, compared to alternative ozone generation methods are given in Table 4. The gaseous output of up to 15 percent ozone by weight (wt%) is high relative to the competing methods. This means that adequate levels of ozone can be dissolved in solution (see Table 4). We anticipate a concentration of 2-5 mg/L can be readily achieved. This concentration cannot be achieved with either CD or UV generation methods. Another key advantage is that the anode chamber, in which the ozone gas is produced, acts as a self-pressurizing chamber. When the output gas line from the generator is connected to a water line, the gas will be generated up to the pressure of that water line, causing the ozone gas to be directly injected into the line without any additional equipment. Also, the ozone injection method does not affect the water pressure of the line. Line pressure is precisely regulated at around 1-1.5 psi for nipple-type bird waters. The injection

system we use will therefore not interfere with the normal operation of the waterer .

Table 4. Comparison of Ozone Generation Processes.

Ozone Source Small Size Systems	Energy (kWh/lb O_3)	Cg (mg/L air)	Cw (mg/L water)
Air Fed Corona 0.5 wt %	30	6.8	2.4
UV Lamp 0.1 wt%	30	1.3	0.5
Electrochemical 12 wt %	25	183*	42.3

*mg/L oxygen

O_3 solubility (C_W) was determined from Henry's law: P = HC, where: P = gas partial pressure above the liquid (mg/L air), H = Henry's law constant (2.59 mg gas/L air per mg gas/L water at 20C), C = concentration of gas in the liquid (mg/L). Much higher dissolved O_3 concentrations are possible with electrochemically generated higher O_3 gas concentrations, assuming Henry's Law relationship is obeyed. In practical situations, C_W is always below the Henry's law prediction due to factors like contacting efficiency. Normally it is difficult to achieve > 2 ppm dissolved ozone using air fed corona discharge units.

(III). Comparison of the Disinfection Capabilities of Electrochemical Versus Corona Discharge Ozone Generators

Corona discharge (CD) is the conventional process for generating ozone gas, but it cannot be used for the type of small scale application described in this proposal. In the corona discharge process, oxygen present in the air, or in an enriched feed gas, is converted from diatomic oxygen (O_2) into ozone (O_3) through an electrical discharge. The air passing into these units must be dried to a dew point of minus 50°C or below. Corona discharge systems do not scale down and there is a price barrier to using CD generators on a small scale. Four of the leading manufacturers of small corona discharge generators are Azco, Purezone, Ozotech and Clearwater Tech. Even the smallest units in the product lines of these companies generate at least 10g/day of ozone, far in excess of the needs of small water feed lines. Ozone generators typically cost $400-450, but they must be used in combination with an air dryer, which itself costs $500-700 depending upon the manufacturer. Also, a method must be used to introduce ozone into the water. A venturi can be used but this is only practical at fast flowing water sources (a venturi uses the water flow to create a negative pressure dissolving ozone in water). If an air pump is used to engage the ozone, the cost will be at least $100 higher again. Therefore, the smallest CD ozone generation system will cost in excess of $1,500 uninstalled.

Small ozone generators (<1 lb/day) also lack the performance necessary to achieve adequate dissolved ozone concentrations. "Industrial scale" CD systems (i.e., those producing 1lb of ozone per day or more) are energy efficient and produce relatively high concentrations of ozone in their output streams (2 wt % for an air-fed corona, or 6 wt % for an pure oxygen-fed corona). However, the smaller versions do not come close to meeting these output concentrations. The significance of being able to generate high ozone concentrations in the gas phase is illustrated in Table 4. The dilute ozone gas streams from CD units cannot easily be engaged into solution, resulting in dissolved ozone concentrations that are too low for many disinfection applications. Finally, in air-fed corona discharge units NOx is formed as a by-product. Nitric acid builds up in the unit. Without frequent (i.e., weekly) maintenance and cleaning these units fail. Furthermore, nitric acid is often formed in the water being treated.

Ozone can also be generated by UV bulbs operating at 185 nm. These systems are, however impractical for water treatment because of their low output concentrations (0.1 wt %).

In summary, electrochemical ozone generators are superior because air drying is not required, the formation of nitric acid is eliminated and, they generate high concentrations of ozone compared to conventional methods for ozone generation.

(IV). Installation and Operation Considerations

This section discusses issues related to how the ozonation method will be operated in a production facility. Two types of watering systems are commonly used in broiler facilities. Nipple drinking facilities are replacing the hanging bell-type waterer. Water supply should be arranged to minimize bird effort in accessing it (May *et al*., 1997). Most hanging bell waterers are forty inches in circumference with the capability of handling up to one hundred birds at one time. Nipple drinkers are spaced about 8 inches apart and generally can handle 15 birds per nipple. Both types of waterers can be hung from a winch system, allowing adjustments as the birds get older and elevation to the ceiling for easy bird catching and litter removal.

The micro-ozone generators will inject ozone into the water lines connecting the waterers and nipple fittings. Attachment to the line will be via a quick connect making removal easy, enabling the devices to be moved to waterers serving other grower houses. We estimate that a 20 mg O_3/hour capacity should be the optimal capacity for the lines feeding the bird waterers. Generator size is determined by the rate of water flow (typically 10L/min) and the ozone residual needed for adequate killing; 1 mg/L is more than sufficient to achieve a high level of disinfection (see Table 2). Therefore, the 20 mg/hr capacity provides a dose sufficient to meet the 10mg/L residual level, with 10 mg/L of excess capacity for O_3 losses that will occur down-stream from the injection point. A number of devices will be placed at intervals along the line to keep the dissolved O_3 levels in the desired range. Each micro ozone generator will produce approximately 100 mL of ozone-containing gas per hour. The gas is introduced into the line through a diffuser for high contacting efficiency. An outlet check valve collects and releases small amounts of excess gas from the line.

The small size of the generators will have minimal environmental impact. Ozone is a toxic gas with a recommended maximum exposure limit of 0.1 ppmV (or 0.04 μg/L). However, broiler facilities are large (>6,000 m^3) and extremely well ventilated, with large air-handling equipment. Under the worst case hypothetical situation, where 6-8 micro-ozone generators were venting all their gaseous output directly into the house rather than into the water line, the ozone levels would not exceed safe limits, even if the ventillation was turned off. There also is little potential for any ozone off gas to emanate from the drinking water. Typically it is impossible to detect any off gas from solutions containing 1 mg/L or less of dissolved ozone.

C3. RELATIONSHIP WITH FUTURE RESEARCH AND DEVELOPMENT

In performing SBIR projects, Lynntech follows a well defined plan of activities to develop a concept and to successfully transition it to a commercial prototype. The goal of Phase I is Proof of Concept, which includes several key elements: (i) articulate the scientific basis; (ii) confirm critical assumptions; and (iii) identify key issues requiring resolution during Phase II. Phase II consists of two major elements. The first one focuses on Technical Feasibility which leads to assembly and testing of a scaled-up laboratory model. The specific features are: (i) resolve major research issues; (ii) establish formulation requirements; (iii) design

formulation process; and (iv) perform definitive testing. The second major element of Phase II activities is Development of a Formulation and Process Prototype. Specific features include: (i) make needed improvements in materials, components, and processes; (ii) establish basis for final scale-up; (iii) optimize product features using models, analyses, and tests; (iv) confirm formulation process; and (v) fabricate prototype or pilot process. The work plan described in this proposal is based on principles, methods, and company policies leading to successful product development.

(I). Mini Ozone Generators: Equipment and Operating Costs

Using our extensive experience in ozone generation and use applications, the cost factors for implementing and using ozone can be realistically defined. Lynntech has a pre-commercial, milligrams/minute electrochemical ozone generation unit. The power required to generate the 20 mg/L of ozone that will be generated in each cell is 0.7 Watts. This low power demand allows the cells to be run for a cost of only a fraction of a cent. The low power consumption allows the ozone generators to operate on AAA or AA rechargeable batteries. It is projected that when the milligrams/minute ozone generation units can be produced in multiples of 10-100 units at a time, the cost will be about $85 each.

This low cost can be achieved because the unit, shown in Figure 3, will only need 5 components for it to operate. Injection molding allows the cell components to be mass produced for little cost while the screw end fittings are available from commercial suppliers and can be bought at low cost when purchased in bulk quantities. The electrode ensemble requires only small amounts of catalyst coated expanded metal and membrane for the cell to operate. The life expectancy of the ozone generation unit, when run on a continuous basis, is a minimum of 5 years. Based on these known factors at the present time, the economics of this technology are extremely favorable.

The use of aqueous ozone to eliminate the contamination in the crop will have many economic benefits to the poultry industry. Eliminating potential pathogens in poultry products will have only positive effects on the broiler retail markets. Through the Phase I feasibility study and Phase II prototype development Lynntech will obtain the intellectual property necessary to push this technology to Phase III and commercial development. Lynntech will work with the necessary broiler industries to fully develop this treatment system. The outcome of this endeavor will make processing poultry safer for the consumer.

C4. PHASE I TECHNICAL OBJECTIVES

The overall objective of this project is to demonstrate the effectiveness of ozonated water, generated under pressure by small, portable electrochemical ozone generators, to decrease or control *Salmonella* bacterial populations in the crops of market age broiler chickens during feed withdrawal. A bench-scale mock-up of the portable electrochemical devices will be plumbed into a research-sized nipple water drinker. Birds will be gavaged with a known amount of *Salmonella* prior to feed withdrawal and the crops and ceca will be evaluated microbiologically.

The work involving the design of the ozone generation device and bird drinking apparatus will be performed at Lynntech, Inc. The apparatus will be moved to the Poultry Science Center at Texas A&M University for experiments involving ozonated water and

market broilers in collaboration with Dr. Billy Hargis.

Experiments planned in the Phase I research and development efforts are designated as four separate and distinct tasks. The tasks in addition to the methods and techniques used are described in detail below. These tasks are designed to answer the following questions:

Will broilers drink water with modest levels of dissolved aqueous ozone?
What ozone concentrations provide an acceptable level of pathogen reduction?
Can these levels of ozone be maintained in nipple watering pipes?

C5. PHASE I WORK PLAN

Task 1. Assembly and Testing of the Broiler Watering System.

The first task will focus on assembly of a test system that will allow for delivery, dissolution and distribution of ozone within the water lines of a nipple drinker. A Lynntech model 724 ozone generator will be made available for these experiments. In Task 1, we will establish ozonation parameters for the watering system to be used in Tasks 2 and 3. This will be accomplished using a laboratory test fixture comprising a length of pipe of the same materials with an internal diameter as the one at the Poultry Science Center (PSC) with a collar and ozone generator-injector attached to one end. The attachment will include diffuser, baffle, gas collector and gas release check valve. The ozone generator will be powered by a variable D.C. power supply. The water line will contain sampling points at increasing distances away from the injection point. Water flow and water pressure will be within the range used at the PSC. Using experimental variables, such as water flow rate, electrolysis current, type of diffuser, etc., will establish how to operate the system in the poultry facility such that ozone levels can be controlled and maintained in the range needed for the Task 2 and 3 studies. By establishing an ozone concentration profile down-stream from the injection site, we can gain an understanding of how far apart the injection sites should be for various operating regimes. The concentration of dissolved ozone will be measured using a Shimadzu (Kyoto, Japan) Model UV 2101 PC double beam spectrophotometer within a flow cell at 254 nm or indirectly by oxidation of indigo blue dye.

We are well aware of the potential hazards associated with the use of ozone (e.g. exposure through inhalation due to off gases). Safeguards to deal with these issues are built into each piece of equipment constructed. Safeguards include the unit enclosures which will be fitted with ozone destruct units to take care of potential leaks. If concentrations are found to exceed expected levels, point source pick-ups with destruct units could be utilized.

Lynntech is well equipped to deal with these or any other ozone issues as they arise. With increasing demand for ozone equipment, Lynntech has been constructing, using and testing safe ozone equipment for more than 5 years. Of which, a great deal of research has gone into perfecting the generation process.

Following these experiments, an appropriately designed system will be assembled at the PSC.

Task 2. Assessment of Bird Acceptability, Palatability of Dissolved Ozone.

The studies in Task 2 will be performed in a test grower house at the PSC through Dr.

Billy Hargis and Dr. David Caldwell, Departments of Veterinary Pathobiology and Poultry Science, Texas A&M University. Seven week-old broilers (n=160) will be obtained from a local commercial grower and placed into four pens giving a commercially-simulated bird density of 40 birds per pen. The pens will be equipped with filtered air and the floors will be covered with wood shavings as litter. All birds will be given a standard broiler ration and water via nipple drinkers *ad libitum* for two days, after which the average pen weights will be recorded. On the third day, the nipple drinkers of three pens will be modified to obtain the following treatments: Pen 1, control (no treatment of water); Pen 2, low dissolved ozone concentration in water (0.1-1 ppm); Pen 3, high concentration of dissolved aqueous ozone (1.0-5.0 ppm), and; Pen 4, water with commercial grade oxygen gas bubbled through at a flow rate approximately equal to the rate of ozone delivery.

For the three weeks that follow, the birds will be evaluated for water consumption by metering the return water feed from the municipal water supply at the test barn. This will be done after the first step-down water pressure regulator so as not to interfere with ozone dissolution. An indirect measurement of water consumption will be made by measuring average in body weight gain (feed conversion) as it is affected by water consumption. Each pen of birds will be weighed at the end of the week (total of 4 times in three weeks). This approach will also allow for determination of any significant water refusal (palatability) issues based on the presence and concentration of ozone. We expect that there will not be any refusal and that ozonation may actually enhance water consumption based on ozone's ability to eliminate off tastes and odors in municipal water supplies.

Task 3. Evaluation of the Disinfection of *Salmonella* in Broiler Crops and Ceca.

(I). Experimental infection with *Salmonella enteritidis*.

A primary poultry isolate of *S. enteritidis*, phage type 13A, will be obtained from the USDA National Veterinary Services Laboratory. This isolate is resistant to the antibiotic novobiocin, No. n-1628 (25 μg/mL) and has been selected for resistance to nalidixic acid, No. n-4382 (20 μg/mL). For these studies, *S. Enteritidis* will be grown according to the method of Lee and Falkow (1990), allowing for attainment of log-phase growth. Cells will be washed three times in distilled water by centrifugation (100 x g) and quantified spectrophotometrically to a stock concentration of approximately 1 x 10^9 cfu/mL in distilled water, using a standard curve generated from comparison of multiple spread platings and optical densities, and then diluted to challenge concentrations (Ramirez *et al*., 1997).

(II). *Salmonella* Recovery from Crops and Ceca.

Commercial broiler chickens (n=160), previously shown to be *Salmonella* free, will be obtained at 6 wk of age from a commercial broiler grower for use in the experiments. For the Task 3 experiments, broilers will be housed in floor pens (18.6 m^2) on new pine shavings in an isolation facility located near the Texas A&M University College of Veterinary Medicine through Dr. Hargis. Broilers will be provided *ad libitum* access to a corn-soybean ration and water for two days. A total of 80 birds will be then be challenged with 1 x 10^8 cfu *S. enteritidis* per milliliter saline by oral gavage.

Table 5. Summary of the Treatment Groups to be Studied in Task 3.

(n=20)	Salmonella challenged?	Aqueous ozone in drinking water?	Feed withdrawal?
Group 1	+	+	+
Group 2	+	+	-
Group 3	+	-	+
Group 4	+	-	-
Group 5	-	+	+
Group 6	-	+	-
Group 7	-	-	+
Group 8	-	-	-

Five days following *Salmonella* challenge, half of the *Salmonella* challenged and half of the control broilers (n=40 each) will be placed on the experimental ozonated water setup in the nipple drinkers (developed and optimized in Task 2) with the remaining half allowed access to the normal nipple drinkers (control). Additionally, half of each pen of birds (n=20) will be randomly selected and subjected to feed withdrawal for 18 h; the remaining birds will continue to have free access to feed. After the 18 h, all birds will be euthanized and the crops and ceca will be collected and plated. A summary of the treatment groups is outlined in Table 5.

Crops will be collected by clamping across the pre and postcrop esophagi using a surgical Carmalt forcep and immersion in boiling water for 1 s to reduce external contamination of the crop. Previous experiments in Dr. Hargis' lab have demonstrated that immersion of crops or ceca in boiling water for 1 s effectively removed all detectable *S. enteritidis* from the surface of intentionally contaminated crops and ceca while not affecting recovery of *S. enteritidis* injected into the lumen of the tissues (data not shown). The crop will be sectioned aseptically below the clamp and the body of the crop, with the lumen and contents exposed, will be collected aseptically in individual Whirl-Pac bags. The ceca will be collected manually by dissection, clamped at the cecal neck, immersed in boiling water for 1 s, and the body of each cecum will be macerated and aseptically collected into sterile Whirl-Pac bags.

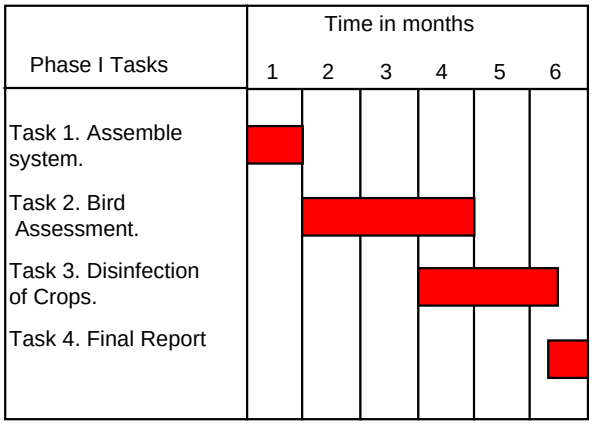

Figure 6 Milestone Chart for the Phase I Effort.

Following crop and ceca removal, 20 mL of tetrathionate broth base, No. 0104-17-6, will be added to each Whirl-Pac bag containing the samples. The samples will be stomached for 30 s and incubated for approximately 24 h at 37°C. Following this enrichment phase, each sample will be individually streaked on brilliant green agar, No. 0285-01-5, plates containing 25 µg novobiocin and 20 mg nalidixic acid/mL to prohibit growth of *Salmonella* other than the antibiotic-resistant challenge isolate. The plates will then be incubated for 24 h at 37°C, examined for the presence or absence of the antibiotic-resistant challenge isolate and enumerated.

A milestone chart plotting the expected progress of the Phase I effort is shown in Figure 6.

C6. RELATED EXPERIENCE

Dr. G. Duncan Hitchens

Dr. Hitchens (P.I., Vice President and Senior Research Scientist) has research and development expertise in both microbiology and ozone technology. Dr. Hitchens has a B.Sc. degree in microbiology and his Ph.D. was in microbial physiology. Dr. Hitchens has directed, or personally carried out, research in electrochemical reactor technology for ozone formation that is directly relevant to the proposed project area. Dr. Hitchens has carried out numerous studies on electrochemical ozone generation. This research has resulted in two patents: "Methods and Apparatus for Using Gas and Liquid Phase Cathodic Depolarizers" United States Patent No.: 5,770,033 and "Method and Apparatus for Electrochemical Production of Ozone", United States Patent No. 5,460,705. The electrochemical process is based on a SPE. A number of R&D projects on PEM water electrolyzers, water treatment devices and hydrogen/oxygen PEM fuel cells have been undertaken at Lynntech, Inc., under Dr. Hitchens' technical management. Since 1990, Dr. Hitchens has conducted or directed several microbiological-related projects. Some of these projects are summarized below.

Disinfection of Salmonella. This was investigated under a USDA contract (USDA Grant Agreement No.: 93-33610-8460). Utilizing an electrochemical ozone generation system, levels of *Salmonella* were reduced two log fold in commercial chicken hatchers using gaseous ozone. Bacteria levels on broiler carcass surfaces were also significantly reduced using ozonated solutions.

Electrochemically Based Modules for Sterilization In the Field. This was investigated for the US Army (Contract No.: DAMD17-91-C-1105). A pilot-scale system was designed, fabricated and tested which demonstrated the effectiveness of gaseous ozone for use as a rapid turn around sterilization method for field hospitals.

Ozone Decontamination and Treatment of Red Bag Medical Waste. A mobile pilot-scale treatment system for the disinfection of red bag waste was field-tested at Lackland AFB, Texas in 1998 for the U.S. Air Force (Contract No.: FY7624-96-C-2001). Gaseous ozone was the disinfectant.

Integrated On-Board Cleaning Process Using Ozone. (Contract No.: NAS9-19447) This contract involved an evaluation of the use of ozone as a cleaning agent and as a laundry disinfectant. A pilot-scale system is being readied for delivery to NASA's Johnson Space Center.

Ozone Sterilization Technique for Endoscopes. (Grant No.: 1R43 E507303) Under this grant, a series of laboratory tests demonstrated the efficacy and effectiveness of ozone as an endoscope disinfection-sterilization agent. The project is currently in Phase II start-up.

Dr. K. Scott McKenzie

Dr. McKenzie (Research Scientist) holds a Ph.D. in toxicology from Texas A&M University. His expertise is in the area of disinfection and oxidation methods for food and water decontamination. His background is very relevant to this project because much of the research he has conducted has involved electrochemical reactors for oxidant synthesis. For instance, Dr. McKenzie has been the lead scientist, first at TAMU and recently at Lynntech, on the use of gaseous ozone for the detoxification of aflatoxin-contaminated corn. This research has resulted in several

publications (see attached Resume). The unique aspect of the research was an electrochemical process was used for the generation of ozone. This reactor was similar in design to the solid polymer electrolyte (SPE) membrane cell described in this proposal. Dr. McKenzie is very familiar with the operation of electrosynthesis reactors for ozone and has first hand knowledge in the testing of these in food decontamination protocols.

Of particular importance to this project is Dr. McKenzie's past employment in a state of the art food processing facility as a production supervisor. During his management training period, he designed, executed and published in house studies that involved (i) identification (Hazard Analysis) of previously undescribed microbial sources of contamination (Critical Control Points) within the various portions of the plant, (ii) description of the extent of contamination from each source through product sampling and subsequent microbiological analysis, and (iii) design, layout and recommendation of intervention strategies to reduce surface contamination. His knowledge of HACCP coupled with his experience using electrochemically generated O_3 to remediate contaminated food and feed make him a key member of the research team.

Additional Technical Expertise

Technical expertise will also be provided by Jim Fyffe who received his B.S. from Texas A&M University in Bioenvironmental Engineering in 1996.

D. KEY PERSONNEL AND BIBLIOGRAPHY

(See Attached Resumes).

E. FACILITITES AND EQUIPMENT

The company occupies 27,000 ft^2 of space which includes general laboratory facilities, analytical chemistry lab, an electronics shop, a basic machining and fabrication facility and two high bay areas where scale-up hardware can be assembled for testing and evaluation. The equipment available to this project includes: LABCONCO Class II Biohazard Cabinet, model 36208-04, Precision Scientific Gravity Convection Incubator, Lab-Line Instruments Adjustable Speed Orbital Shaker, model 4625, Carl Zeis Compound Microscope, Tuttnauer / Brinkmann Autoclave, model number 2540E, Pipettemen, spreaders, burners, plates and various media. Also, Lynntech will be installing a Waters *Integrity* LC-MS system in September of 1998. Other apparatus includes: power supplies, potentiostats, X-Y recorders, a Varian, atomic absorption spectrophotometer, Model AA-875, a Shimadzu UV/Visible spectrophotometer, Model UV 2101 PC, a Dionex ion-chromatograph, Model DX-100 and a "Nanopure" ultrapure water system. In addition, standard laboratory equipment, such as glassware, pH-meters, voltmeters, balances, fume hoods and computers and computer network consisting of over 70 IBM and Macintosh personal computers are available. The Product Development area is equipped with CAD capabilities for developing comprehensive engineering drawings and

electronic schematics. Basic machining, drilling, metal cutting, bending and welding can be performed as needed. Numerous tools for mechanical assembly and testing are also available and Lynntech personnel fabricate all types of electrical wiring harnesses and connectors.

F. OUTSIDE SERVICES

Dr. Billy Hargis and Dr. David Caldwell will provide consulting as experts in the field of reduction and control of pathogens on poultry. Dr. Caldwell will assist with Tasks 3 objectives through retrofitting of the ozonation apparatus developed in Tasks 1 and 2 at a research broiler house currently under his supervision. Dr. Hargis will provide acquisition and gavaging of broilers and subsequent microbiological analysis of crop and cecal microbes in his laboratory. These studies will be conducted in part at the Poultry Science Center on the campus of Texas A&M University under the direct supervision of Professors Hargis and Caldwell, Departments of Poultry Science and Veterinary Pathobiology, Texas A&M University. Letters from Drs. Hargis and Caldwell acknowledging their collaborative arrangement and participation in this project are attached.

G. SATISFYING THE PUBLIC INTEREST

Salmonella contamination of broiler products is a continual problem for the poultry industry. With the phasing out of chlorine related products in the broiler cleaning phase there is more room for innovative technologies such as ozone generators to be used in their place. The GRAS status that ozone has places it in a strong position to dominate the food treatment industry. Lynntech's new technology will fill a gap that is missing in the current broiler treatment process allowing for greater removal of potential pathogenic species from the final store ready product. The improved quality of the product will ultimately be passed on to the consumer which can only benefit the poultry industry.

H. POTENTIAL POST APPLICATIONS

(I). Company Information

Lynntech, Inc., is a small business specializing in technology development. The company has a staff of 65 employees of which 23 are at the Ph.D. level. In addition to being successful in developing new concepts having federal government and industrial potential, we have a record of moving ideas from the laboratory proof-of-concept stage to the pilot scale hardware system. Research and development, testing, engineering design and fabrication are all performed in-house using our team of multi-disciplinary staff. Our small size permits high intensity efforts to be carried out in rapid succession.

The business objectives of Lynntech, Inc. have the development and commercialization of electrochemically based technologies for their foundation. The company has strong R&D capabilities in the area of electrochemical technologies. In addition to federal government grants and contracts, the company has secured R&D contracts from industrial corporations, and provides consulting services to private industry. Arising from previous and existing contracts, the company has acquired the services of key internationally renowned

consultants and developed subcontracting relationships with research centers at Texas A&M University.

(II). General Appraisal of the Marketplace

Ozone oxidation allows commercial entities, that need water purification, to use ozone more cost effectively as a purifying agent. The actual size of the water treatment markets are listed in Table 6.

Table 6. Potential Markets and Market Sizes for Ozone Based Technologies.

Potential Market	Market size
Water purifying/cleaning compounds	$267 million
Oxidizing and bleaching agents	$2.70 billion
Liquid detergents	$1.70 billion
Powder detergents	$2.20 billion
Sludge management	$1.98 billion
Industrial waste/wastewater	$4.53 billion
Municipal water/wastewater	$4.83 billion
Bottled water industry	$3.00 billion

The electrochemical ozone generator and a highly sensitive ozone monitor has implications in a wide variety of industries. Some of these industries are water treatment, electronics, pharmaceutical, food and beverage, environmental remediation and electricity generation. It can also be used to purify the water used in aquariums, laboratories, chemical processing, and laundry applications.

It is projected that this technology can be rapidly transferred to industrial and consumer-based products. No technical obstacles to commercial manufacturing and marketing are foreseen and Lynntech has developed a strategic partnership with Teledyne Water Pik to bring this technology to the market place. A world wide marketing survey made by Water Pik identified 5 major markets outside of the United States where new small scale, self-contained water treatment units have extensive market potential. The information gained from the Phase I research will have significant implications on the commercialization prospects of the ozone generator.

The commercialization efforts will be made during the second year of the Phase II. Initial patents will be submitted to establish intellectual property ownership which is essential for all subsequent steps in the commercialization plan. Lynntech, Inc., typically prosecutes between 5 and 10 patents per year using its internal resources. We will solicit interest from industry by disclosure of inventions (and experimental data) resulting from Phase II research; non-disclosure agreements will be used where appropriate. To accelerate the commercialization process, Lynntech has created the position of Manager, Marketing and Sales, within it's organizational structure in late 1996. This position has recently been filled by Thomas D. Rogers, who has a strong technical background and expertise in marketing and sales. Part of his role within the company will be to market SBIR developed technologies to various industrial concerns, government agencies and government prime contractors. Through his activities, securing Phase III follow-on funding commitments for SBIR projects will be greatly enhanced.

Lynntech has been, and is presently, aggressively pursuing a Phase III follow-on funding commitment for this project from interested industrial concerns. As of the time of writing this document, a Phase III commitment had not been secured. However, it is anticipated that such a commitment will be obtained either from one company, or a consortium of companies, over the next few months.

I. CURRENT AND PENDING SUPPORT

(No similar proposals have been submitted).

Resume: **DR. G. DUNCAN HITCHENS (SENIOR RESEARCH SCIENTIST)**

EDUCATION:

Ph.D.: Microbial Physiology; Department of Botany and Microbiology, University College of Wales, Aberystwyth, Wales (1985).

B.Sc.: Microbiology; Department of Botany and Microbiology, University, College of Wales, Aberystwyth, Wales (1981).

EMPLOYMENT:

Vice President, Lynntech, Inc., College Station, Texas, 1991-Present

Senior Scientist, Lynntech, Inc., College Station, Texas, 1989-91

Research Associate, Center for Electrochemical Systems and Hydrogen Research, Texas A&M University, College Station, Texas, 1988-89

Research Associate, Laboratory of Surface Electrochemistry, Department of Chemistry, Texas A&M University, College Station, Texas, 1985-88

PUBLICATIONS: 35 PRESENTATIONS & ABSTRACTS: 53 PATENTS: 4

SELECTED PUBLICATIONS:

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Resume: **DR. K. SCOTT MCKENZIE**

EDUCATION:

Ph.D.: Toxicology, Texas A&M University, (1993-1997)

B.S. : Biomedical Science, College of Veterinary Medicine, Texas A&M University (1987-1991) Animal Science, College of Agriculture and Life Sciences, Texas A&M University (1987-1991)

EMPLOYMENT:

Research Scientist, Lynntech, Inc., College Station, Texas, 1997 to present.

Graduate Research Assistant, Faculty of Toxicology, Department of Veterinary Public Health, Texas A&M University, 1993-1997.

Production Supervisor, Cargill Corp., EXCEL Division, Fort Morgan, Colorado, 1991-1993.

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Education:

B.S.	*University of Minnesota*	1980	D.V.M.	*University of Minnesota*	1986
M.S.	*University of Georgia*	1983	Ph.D.	*University of Minnesota*	1987

Professional Organizations and Honors:

American College of Poultry Veterinarians (Diplomate) **1992**-Present
Poultry Science Association & American Veterinary Medical Association
Texas Veterinary Medical Association
Carrington Laboratories Faculty Award for "Outstanding Research Program in Cell Biology" (**1991**)
Texas Poultry Improvement Board: Advisor (**1990**-Present)
Poultry Disease Diagnostic Laboratory: "Director" (**1987** - Discontinued September 1, 1991)
Recipient of the 1993 Poultry Science Association Research Award
Recipient of USDA/ARS Certificate of Merit for Scientific Leadership **1994**
Center for Food Safety Member (**1995**-Present)
Vice President, Southern Poultry Science Society, **1996-1997**
President, Southern Poultry Science Society, **1998**
The **1998** National Broiler Council Research Award, Poultry Science Association

Selected Recent and Relevant Publications:

Barnhart, E.T., D.J. Caldwell, M.C. Crouch, J. A. Byrd, D.E. Corrier, and B.M. Hargis, 1998. Evaluation of Potential Disinfectants for Pre-Slaughter Broiler Crop Decontamination. Poultry Sci. (submitted).

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Barnhart, E.T., D.J. Caldwell, M.C. Crouch, J. A. Byrd, D.E. Corrier, and B.M. Hargis, 1998. Effect of Lactose Administration in Drinking Water Prior to and During Feed Withdrawal on *Salmonella* Recovery From Broiler Crops and Ceca. Poultry Sci. (submitted).

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Resume: **DAVID J. CALDWELL**

Education: B.S (Poultry Science) Texas A&M University, 1991
M.S. (Veterinary Microbiology) Texas A&M University, 1994
Ph.D. (Veterinary Microbiology) Texas A&M University, 1997

Title: Assistant Professor, Departments of Poultry Science, College of Agriculture and Life Sciences, and Veterinary Pathobiology, College of Veterinary Medicine, Texas A&M University

Professional Organizations:

-American Association for the Advancement of Science
-World's Poultry Science Association
-American Association of Veterinary Immunologists
-Poultry Science Association
-Society for Leukocyte Biology
-The American Society for Microbiology

Selected Scientific Publications:

Caldwell, D.J.., B.M. Bargis, D.E. Comer, J.D. Williams, L. Vidal, and l.R. DeLoach, 1994. Predictive value of multiple drag-swab sampling for the detection of *Salmonella* from occupied or vacant houses. *Avian Vis.* 38:461-466.

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TEXAS A&M UNIVERSITY
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August 28, 1998

Dr. Scott McKenzie
Lynntech, Inc.
7610 Eastmark Dr., Ste. 105
College Station, TX 77840

Dear Dr. McKenzie:

As per our discussions during the last several months, I am pleased to assist and provide advise as needed for your project under development for consideration by USDA/SBIR involving control of orally-transmitted infectious and zoonotic diseases using electrochemically-generated ozone in drinking water. Recently, our laboratory has demonstrated that feed withdrawal is associated with tremendous increases in contamination of the upper gastrointestinal tract of chickens with important human pathogens such as *Salmonella, Campylobacter* and *E. coli* and that this area of the intestinal tract is critical for control of contamination of commercially processed poultry carcasses. In addition to potential reductions in these contaminants important to food safety, we should expect that effective concentrations of ozone may reduce bird-to-bird transmission of infectious agents important to production efficiency and poultry health. Based on comparative research, it would also appear that this technology may indeed provide an attractive and effective solution to these problems.

As we discussed, my laboratory is prepared to offer you any and all technical assistance that you may require in these investigations. We are experienced with research investigations relating to disinfection of drinking water and have available marked strains of *Salmonella, Campylobacter* and *Listeria* for your use. As we discussed, I can offer you access to our experimental poultry facilities at the Texas A&M University Poultry Science Research Center and can assist you with field research endeavors with several commercial poultry companies. I enthusiastically support your efforts in this area and am anxious to begin this cooperative effort.

Sincerely,

B.M. Hargis, DVM, PhD
Professor

TEXAS A&M UNIVERSITY
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Room 101 Kleberg Animal and Food Science Center
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September 2, 1998

Dr. K. Scott McKenzie
Lynntech, Inc.
7610 Eastmark Drive, Suite 105
College Station, TX 77843

Dear Dr. McKenzie

I am writing this letter to confirm my willingness to serve as a collaborator and research team member on your USDA/SBIR proposal entitled "A New Technique for Ante-Mortem Control of Pathogens in Broilers". The extent and severity of *Salmonella* and *Campylobacter* contamination in the broiler industry, especially during feed withdrawal, has mandated the development of new technologies for pathogen reduction. The use of ozone in the drinking water of market broilers should provide an environmentally friendly alternative to other intervention methods and should compliment current approaches to pathogen control.

I can provide research facilities at the Texas A&M University Poultry Science Center for the Task 2 studies involving water consumption of ozonated water as outlined in the Proposal. I am looking forward to working with Lynntech on this project and hope your proposal meets with successful reviews.

Sincerely,

David J. Caldwell, Ph.D.
Assistant Professor
Departments of Poultry Science and
Veterinary Pathobiology
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