GRAS Notice (GRN) No. 488
http://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/default.htm

ORIGINAL SUBMISSION

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September 6, 2013

Paulette Gaynor, Ph.D.
Deputy Division Director
Office of Food Additive Safety (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740

RE: **Benzalkonium chloride as a component of Free N Clear™ GRAS Notification**

Dear Dr. Gaynor:

In accordance with proposed 21 CFR § 170.36 (a notice of a claim for exemption based on a GRAS determination) published in the Federal Register (62 FR 18937-18964), I am submitting in triplicate, as the agent of the notifier, Marvel Technologies USA, LLC, 1224 Columbia Avenue, Franklin, TN 37064, a GRAS notification for the use of benzalkonium chloride (BAC) as a component of Free N Clear™ (FNC) as a processing aid for lettuce and carrot products. FNC will be diluted to a 2% solution (2% FNC) for this purpose. Such use could potentially increase daily dietary (aggregate) exposure to BAC to 0.0530 mg/kg bw/day. A GRAS expert panel dossier, setting forth the basis for the GRAS determination, as well as *curriculum vitae* of the members of the GRAS panel, are enclosed.

Best regards,

(b) (6)

19Sept13

George A. Burdock, Ph.D., DABT, FACN
Diplomate, American Board of Toxicology
Fellow, American College of Nutrition

1. GRAS Exemption Claim

A. Claim of Exemption from the Requirement for Premarket Approval Pursuant to Proposed 21 CFR 170.36(c)(1)

Marvel Technologies USA, LLC, on the advice of qualified experts, has determined benzalkonium chloride (BAC) to be generally recognized as safe (GRAS) as a component of Free N Clear™ (FNC) as a processing aid for lettuce and carrots, under the conditions of its intended use as described below. The basis for this finding is described in the following sections.

Signed,

(b) (6)

Date 19 Sept 2013

George A. Burdock, Ph.D., DABT, FACN
Diplomate, American Board of Toxicology
Fellow, American College of Nutrition
859 Outer Road
Orlando, FL 32814

000032

(i) Name and Address of the Notifier

Jack A. Wheeler
Marvel Technologies USA, LLC
1224 Columbia Avenue
Franklin, TN 37064

Agent of the Notifier:

George A. Burdock, Ph.D., DABT, FACN
Diplomate, American Board of Toxicology
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(ii) Common Name of the Notified Substance

The common name of benzalkonium chloride, for the purposes of this GRAS Notification has been defined as:

Benzalkonium chloride

(iii) Conditions of Use

Benzalkonium chloride will be used as a component of Free N Clear™ (FNC), which is used as a processing aid for lettuce and carrots when diluted to a 2% solution (2% FNC solution). Such use could potentially increase daily dietary (aggregate) exposure to BAC to 0.0530 mg/kg bw/day.

(iv) Basis of GRAS Determination

Pursuant to 21 CFR § 170.3, benzalkonium chloride, as component of Free N Clear™ (FNC), has been determined GRAS by scientific procedures for its intended conditions of use. The safety of benzalkonium chloride for this use is supported by subchronic (OECD[1] Guideline No. 408) and genotoxicity studies (OECD Guideline No. 471 and 474) on 2% FNC solution and acute, subchronic, chronic, and reproductive/developmental toxicity studies on benzalkonium

[1] Organisation for Economic Co-operation and Development

chloride.[2] This determination is based on the views of experts who are qualified by scientific training and experience to evaluate the safety of substances used as ingredients in food.

(v) Availability of Information

The data and information that serve as a basis for this GRAS determination are available for FDA review and copying at reasonable times at:

Burdock Group
859 Outer Road
Orlando, FL 32814
Telephone: 407-802-1400
Facsimile: 407-802-1405
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Alternatively, data and information that serve as a basis for this GRAS determination may be sent to FDA upon request.

2. Detailed Information about the Identity of the Notified Substance

A. Identity

Benzalkonium chloride (BAC) (Figure 1) is a member of the class of quaternary ammonium compounds. It is a mixture of alkylbenzyl dimethylammonium chlorides of the general formula $[C_6H_5CH_2N(CH_3)_2R]Cl$, with the R group including *n*-octyl (*n*-C_8H_{17}; C8) and extending through higher homologues. The average molecular weight of BAC is 360. BAC has been assigned a number of different CAS numbers, depending on the number of carbons in the R groups. In United States Pharmacopeia (USP)-grade benzalkonium chloride (USP-BAC; CAS No. 8001-54-5), the R groups of *n*-$C_{12}H_{25}$, *n*-$C_{14}H_{29}$ and *n*-$C_{16}H_{33}$ (C12, C14 and C16) predominate, with the total amount of the C12 and C14 homologs not less than 70.0% of the total alkylbenzyl dimethylammonium content.

[2] References for these studies are provided in attached " Dossier in Support of the Generally Recognized as Safe (GRAS) Status of Benzalkonium Chloride as a Component of Free N Clear™ as a Processing Aid for Lettuce and Carrots", dated August 6, 2013.

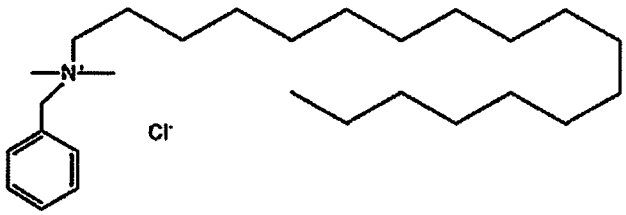

Figure 1. Structure of benzalkonium chloride (BAC)

USP-BAC is an ingredient of Free N Clear™ (FNC), a proprietary mixture.[3] The concentrated form of FNC is prepared from mixing specified amounts of USP-BAC solution (50%), methyl paraben, acetic acid and water. The three ingredients of FNC act together – BAC is a quaternary ammonium cationic surfactant which exhibits antimicrobial activity towards a wide variety of bacteria, methyl paraben is an adjuvant which may act in combination or synergism with other antimicrobial agents, and acetic acid is a weak organic acid that helps maintain pH of FNC at the desired level. FNC concentrate is diluted 1:50 (2% FNC solution) for use as a processing aid for lettuce and carrots.

BAC is approved for use as a component of adhesives for food packaging[4] and for several non-food uses. BAC-containing formulations that are added directly to water[5] or used to treat hard nonporous surfaces, wood, plants or turf, are regulated as pesticides. FNC (or the BAC component of FNC) is not subject to regulation as a pesticide when used in processing plants as a processing aid for lettuce or carrots, as it will not be used on a pest.[6,7]

Common or Usual Name:

The common name or usual name is "benzalkonium chloride". Benzalkonium chloride is also known as BAC, alkyl dimethyl benzyl ammonium chloride (ADBAC) or alkylbenzyl dimethylammonium chloride.

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[3] Free N Clear™ has been trademarked as Free N Clear Solutions® (personal correspondence from Jack Wheeler, Marvel Technologies USA, LLC, September 5, 2013).
[4] Title 21 of the US Code of Federal Regulations (CFR), section §175.105, 2010. Approved substance is Alkyl (C10-20) dimethylbenzyl ammonium chloride.
[5] Swimming pools, decorative ponds/fountains, spas, cooling water towers, oil field drilling muds and packing fluids, small process water systems, humidifiers, and cut flower applications.
[6] Personal correspondence from Dennis Edwards, US Environmental Protection Agency, May 6, 2013.
[7] Title 40 of the US Code of Federal Regulations (CFR), section §152.5.

B. Method of Manufacture

FNC is manufactured at ambient temperature by combining food grade glacial acetic acid, food grade methyl paraben and USP-BAC[8] (50% solution) in precise amounts and in a defined sequence.

C. Specifications

As mentioned above, the BAC component of FNC meets USP specifications. USP-BAC solution contains 50% BAC (CAS No. 8001-54-5) and may contain up to 10% alcohol. The remainder is water. In USP-BAC, the R groups of *n*-$C_{12}H_{25}$, *n*-$C_{14}H_{29}$ and *n*-$C_{16}H_{33}$ (C12, C14 and C16) predominate, with the total amount of the C12 and C14 homologs not less than 70.0% of the total alkylbenzyl dimethylammonium content.

The permissible amount of BAC in FNC ranges from 2638-2777 ppm. Measured concentrations of BAC in 1:50 dilutions (2% solutions) of lots of FNC meeting specifications average 74 ppm.

D. Utility

The concentrated FNC will be diluted 1:50 by the user (producing 2% FNC solution) prior to application to carrots or lettuce in processing facilities. Users will be directed to submerge carrots or lettuce in 2% FNC solution (as incorporated into the wash solution) for five minutes. A 5-minute exposure to 2% FNC solution effectively kills *Listeria monocytogenes*, *Escherichia coli* 0157:H7 and *Salmonella typhimurium* in solution and *E. coli* on lettuce and carrots (APPENDIX 1).

3. Self-Limiting Levels of Use

FNC contains specified amounts of USP-BAC, methyl paraben and acetic acid and is recommended for use at a 1:50 dilution (2% FNC solution). It is possible that users may dilute FNC to a concentration greater than 2% or submerge produce for longer than five minutes. However, based on cost, users are expected to use the minimal concentration of FNC and immersion time for the desired effect. Consumption of BAC is expected to be below the

[8] The BAC solution is USP grade. It contains 50% BAC (CAS No. 8001-54-5) and may contain up to 10% alcohol. The remainder is water.

conservatively estimated 90th percentile level of intake, as (a) the immersion time used to estimate BAC consumption from use of 2% FNC solution on lettuce and carrots was three hours and (b) the estimated 90th percentile level of intake assumes 90th percentile intake of BAC from current sources. As shown in APPENDIX 1, increasing exposure time from 5 to 15 minutes does not improve the efficacy of 2% FNC solution.

4. Basis of GRAS Determination

The determination that benzalkonium chloride is GRAS is on the basis of scientific procedures, as outlined in the attached " Dossier in Support of the Generally Recognized as Safe (GRAS) Status of Benzalkonium Chloride as a Component of Free N Clear™ as a Processing Aid for Lettuce and Carrots", dated August 6, 2013. On the basis of the data and information described in the attached dossier and other publicly available information, there is consensus among experts qualified by scientific training and experience to evaluate the safety of substances added to food, that there is reasonable certainty that benzalkonium chloride is GRAS under the intended conditions of use.

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APPENDIX 1 –Utility of 2% FNC solution

The ability of 2% FNC solution to inhibit growth of three different strains of *Listeria monocytogenes* (*Listeria*), *Escherichia coli* 0157:H7 (*E. coli*) or *Salmonella typhimurium* (*Salmonella*), either alone or in combination (pathogen cocktail) was tested by Daniel Fung, Professor of Food Science, Kansas State University, Manhattan Kansas. For the experiments that examined the efficacy against the individual bacteria, the starting concentrations of *Listeria*, *E. coli* and *Salmonella* were approximately 1 x 10^7 Colony Forming Units (CFU)/ml, 1 x 10^7 CFU/ml and 1 x 10^6 CFU/ml, respectively. For the pathogen cocktail experiment, the starting concentrations of *Listeria*, *E. coli* and *Salmonella* were approximately 1 x $10^{6.5}$ CFU/ml, 1 x 10^6 CFU/ml and 1 x $10^{4.5}$ CFU/ml, respectively. Aliquots (1 ml) of each bacterium (or the pathogen cocktail) were added to bottles containing 99 ml of 2% FNC solution. Aliquots (1 ml) from each bottle were collected as quickly as possible (0 seconds) and at 10, 15, 30, 60 and 300 seconds (5 minutes) after addition of each bacterium (or the pathogen cocktail) and plated on agar. All agar plates were incubated at 35°C for 24 hours and colonies of the specific bacterial populations were counted. Results of each experiment are shown in Figures 2-5 below. The results show that 2% FNC solution produced a 5.5 log reduction in *Listeria* within five minutes, 4-5 log reductions in *E. coli* and *Salmonella* and 3-5 log reductions of the pathogens in the pathogen cocktail within five minutes, indicating that a five minute exposure to 2% FNC solution effectively kills *Listeria*, *E. coli* or *Salmonella*, alone or in combination. The efficacy of 2% FNC solution is similar to other produce sanitizers.[9]

[9] Lopez-Galvez, F., Allende, A., Selma, M.V., Gil, M. I. (2009) Prevention of *Escherichia coli* cross-contamination by different commercial sanitizers during washing of fresh-cut lettuce. *International Journal of Food Microbiology* 133:167-171.

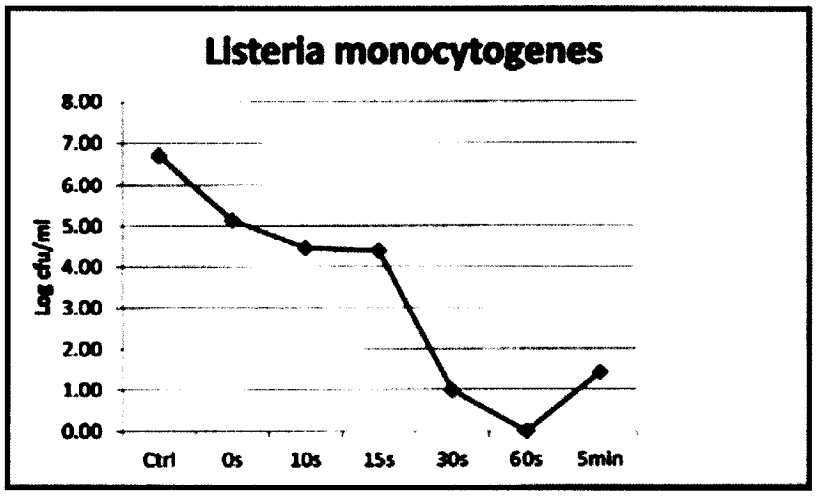

Figure 2. Effect of 2% FNC solution on *Listeria monocytogenes*

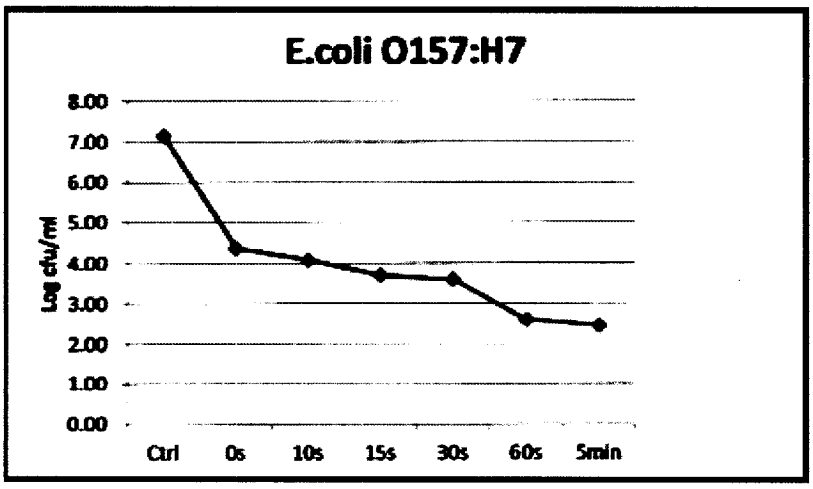

Figure 3. Effect of 2% FNC solution on *Escherichia coli* 0157:H7

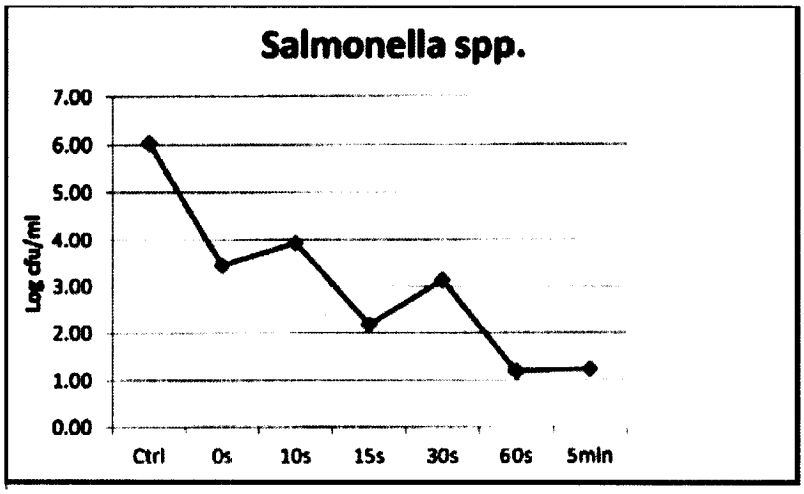

Figure 4. Effect of 2% FNC solution on *Salmonella typhimurium*

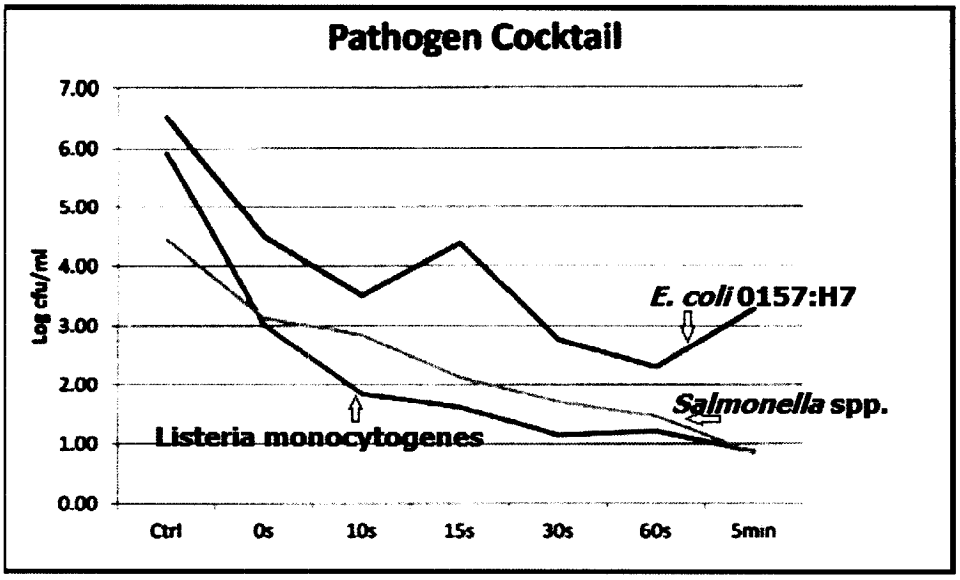

Figure 5. Effect of 2% FNC solution on *Listeria monocytogenes*, *Escherichia coli* 0157:H7 and *Salmonella typhimurium* in combination

The ability of 2% FNC solution to kill *E. coli* on lettuce and carrots was tested by Microbac Laboratories Inc., Nashville, TN according to a standard operating procedure based on the 3M™ Petrifilm *E. coli*/coliform method. Four stalks of romaine, organic lettuce and four full size organic carrots were used in the test. Carrots were cut into 2-inch sections. Leaves of lettuce or 2-inch sections of carrots were held with forceps and swabbed with *E. coli* (72,000 CFU[10]/mL ATCC #8739) using a 3M™ Swab-Sampler with Neutralizing Buffer (4-mL size) at ambient temperature. After 5 - 15 minutes, the full face of each lettuce leaf and each carrot piece were swabbed with a clean swab to obtain a baseline measurement for the amount of bacteria. Each produce sample was then placed in its own container (4 ounce for carrots and 8 ounce for carrots). 2% FNC solution was then placed into each container to the point that the produce was submerged in the FNC solution. After five minutes, two samples of lettuce and two samples of carrots were removed from the containers and swabbed with clean swabs. The other two samples of lettuce and carrots were swabbed with clean swabs after a 15 minute exposure to 2% FNC solution. Swabbing was performed consistently and all swabs were placed back into their respective tubes containing buffer. Tubes were shaken vigorously to release bacteria from the swab into the medium. The medium from each tube was then poured into separate 3M Petrifilm *E. coli*/coliform count plates. Plates were incubated for 48 ± 2 hours at 35 ± 1 °C. Colonies were counted using a Darkfield Quebec Colony Counter from American Optical Corporation. The results of the test are shown in Table 1. After a five minute exposure, amounts of *E. coli* on lettuce and carrots were reduced by 98.4% and 91.2%, respectively. These results show that a five minute dip in 2% FNC solution effectively kills *E. coli* on lettuce and carrots. The efficacy of 2% FNC solution in reducing *E. coli* on lettuce (approximately 2 logs after five minutes) is similar to that of other produce sanitizers.[9]

Table 1. Efficacy of 2% Free N Clear™

	E. coli (CFU/swab)*				
Produce	**0 min**	**5 min**	**% Reduction**	**15 min**	**% Reduction**
Lettuce	950 ± 189	15 ± 5	98.4	55 ± 25	94.2
Carrots	570 ± 70	50 ± 20	91.2	180 ± 20	68.4

*Data are presented as mean ± standard deviation; CFU = colony forming unit; min = minutes.

[10] CFU = colony forming units

DOSSIER IN SUPPORT OF THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF BENZALKONIUM CHLORIDE AS A COMPONENT OF FREE N CLEAR™ AS A PROCESSING AID FOR LETTUCE AND CARROTS

August 6, 2013

FINAL

Panel Members

Douglas L. Archer, Ph.D.

Ed Carmines, Ph.D.

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DOSSIER IN SUPPORT OF THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF BENZALKONIUM CHLORIDE AS A COMPONENT OF FREE N CLEAR™ AS A PROCESSING AID FOR LETTUCE AND CARROTS

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DOSSIER IN SUPPORT OF THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF BENZALKONIUM CHLORIDE AS A COMPONENT OF FREE N CLEAR™ (FNC) AS A PROCESSING AID FOR LETTUCE AND CARROTS

1. EXECUTIVE SUMMARY

The undersigned, an independent panel of recognized experts (hereinafter referred to as the Expert Panel),[1] qualified by their scientific training and relevant national and international experience to evaluate the safety of food ingredients, was requested by Marvel Technologies USA, LLC (hereinafter referred to as Marvel) to determine the Generally Recognized As Safe (GRAS) status of benzalkonium chloride (BAC) as a component of Free N Clear™ (FNC) as a processing aid for lettuce and carrot products, based on scientific procedures. FNC is a proprietary mixture composed of BAC, acetic acid, and methyl paraben in an aqueous base. Both acetic acid and methyl paraben are safe and suitable ingredients already approved for use in foods, therefore the focus of this dossier is on the safety-in- use of BAC as a component of a 2% FNC solution. A 2% solution of FNC is to be used as a processing aid on lettuce and carrots. Such use could potentially increase daily dietary (aggregate) exposure to BAC to 0.0530 mg/kg bw/day. Marvel assures that all relevant, unpublished information in its possession related to the safety of FNC has been supplied to Burdock Group and has been summarized in this dossier. A comprehensive search of the scientific literature was conducted through March 1, 2013 for safety and toxicity information on FNC, BAC and related substances, and all relevant information has been summarized in this dossier. That information, along with supporting documentation, was made available to the Expert Panel. In addition, the Expert Panel independently evaluated materials deemed appropriate and necessary. Following an independent, critical evaluation, the Expert Panel conferred and unanimously agreed that BAC is safe for use as an ingredient in FNC, when a 2% solution of FNC is used as a processing aid on lettuce and carrots.

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[1] Modeled after that described in Section 201(s) of the Federal Food, Drug, and Cosmetic Act, as amended. See also attachments (*curriculum vitae*) documenting the expertise of the Panel members.

2. INTRODUCTION

Free N Clear™ (FNC) is a proprietary mixture[2] composed of benzalkonium chloride (BAC), acetic acid, and methyl paraben in an aqueous base. The concentrated form of FNC is prepared from mixing specified amounts of United States Pharmacopeia (USP)-grade benzalkonium chloride (BAC) solution (50%) (USP, 2010), methyl paraben, acetic acid and water. This concentrate is diluted 50:1 (2% FNC solution) for use. A 2% FNC solution contains 96 - 100 ppm (0.01%) USP-BAC. The intended use of 2% FNC is as a processing aid on lettuce and carrots. The safety in use of BAC as a component of a 2% FNC solution is the primary focus of this dossier, as acetic acid and methyl paraben are safe and suitable ingredients already approved for use in foods. This dossier is a summary of the scientific evidence that supports the general recognition that the residual amount of BAC on lettuce and carrots after use of a 2% FNC solution as a processing aid is safe for human consumption.

2.1. Description

Free N Clear™ (FNC) is a clear, colorless solution prepared from mixing 118 g of USP-grade benzalkonium chloride (BAC) solution (50% concentration), 236 g of acetic acid and 118 g of methyl paraben (synonym: methyl *p*-hydroxybenzoate or 4-hydroxybenzoic acid, methyl ester) and adding water to 6.3 gallons. The structures of BAC (CAS No. 8001-54-5),[3] acetic acid (CAS No. 64-19-7) and methyl paraben (CAS No. 99-76-3) and are shown in Figure 1. The three ingredients of FNC act together– BAC is a quaternary ammonium cationic surfactant which exhibits antimicrobial activity towards a wide variety of bacteria (Rowe, *et al.*, 2006), methyl paraben is an adjuvant which may act in combination or synergism with other antimicrobial agents (Rowe, *et al.*, 1996), and acetic acid is a weak organic acid that helps maintain pH of FNC at the desired level. FNC will be used as a processing aid applied to lettuce and carrots at a concentration of up to 2% in water.

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[2] A U.S. federal trademark registration was submitted on September 10, 2012 for Free N Clear™.
[3] CAS = Chemical Abstracts Service

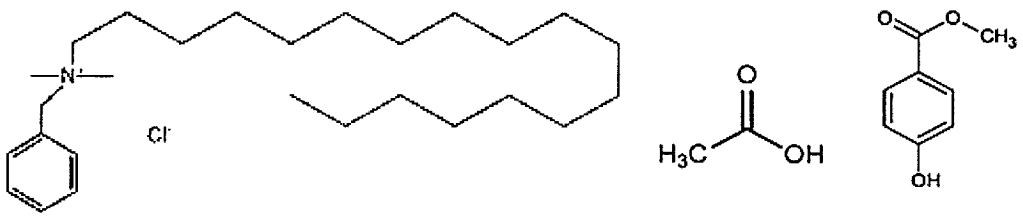

Figure 1: Structures of benzalkonium chloride (BAC), acetic acid, and methyl paraben, respectively.

BAC a member of the class of quaternary ammonium compounds, and may be referred to as alkyl dimethyl benzyl ammonium chloride (ADBAC) or alkylbenzyl dimethylammonium chloride. It is a mixture of alkylbenzyl dimethylammonium chlorides of the general formula $[C_6H_5CH_2N(CH_3)_2R]Cl$, with the R group including *n*octyl (n-C_8H_{17}; C8) and extending through higher homologues. The average molecular weight of BAC is 360 (Rowe, *et al.*, 2006). BAC has been assigned a number of different CAS numbers, depending on the numbers of carbons in the R groups. In USP-BAC (CAS No. 8001-54-5), the R groups of n-$C_{12}H_{25}$, n-$C_{14}H_{29}$ and n-$C_{16}H_{33}$ (C12, C14 and C16) predominate, with the total amount of the C12 and C14 homologs not less than 70.0% of the total alkylbenzyl dimethylammonium content (USP, 2010).

Acetic acid is a directly added food ingredient affirmed as GRAS for use in human food, with its level of use limited only by current good manufacturing practice (cGMP).[4] Maximum levels of use of acetic acid that are consistent with cGMP are 9.0% for condiments and relishes, 3.0% for gravies and sauces, 0.8% for cheeses and dairy product analogs, 0.6% for meat products, 0.5% for fats, oils, and chewing gum, 0.25% for baked goods, and 0.15% for all other food categories.[4] Acetic acid is also approved for use as an indirect food additive in sanitizers used to control the growth of microorganisms, with the level of use limited only by cGMP.[5,6] As the concentration of acetic acid in a 2% solution of FNC (0.0239%, see below) is encompassed by the FDA GRAS affirmation for acetic acid, which permits use of up to 0.15% acetic acid in

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[4] Title 21 of the US Code of Federal Regulations (CFR), section §184.1005.
[5]Title 21 of the US Code of Federal Regulations (CFR), section §178.1010.
[6]21 CFR §178.1010 does not include use of acetic acid alone or in combination with ingredients other than hydrogen peroxide, peracetic acid, sulfuric acid, and peroxyoctanoic acid. The regulation mentions specific concentrations of each of these substances that should be contained in the sanitizing agents.

foods such as produce, the use of acetic acid as a component of FNC for use as a processing aid for lettuce and carrots is considered GRAS by reference. Therefore, in this dossier, information about acetic acid will be limited to a discussion of the level in FNC.

Methyl paraben is widely used as a preservative[7] in food products, cosmetics and over-the-counter (OTC) pharmaceutical formulations (Soni *et al.*, 2002). It can be used alone or in combination with other parabens, or with other antimicrobial agents. Methyl paraben is GRAS when used as a chemical preservative in foods at a limit of 0.1% (1000 ppm)[8] under cGMP (21 CFR §184.1490). As the concentration of methyl paraben in a 2% solution of FNC is encompassed by the GRAS affirmation for methyl paraben, methyl paraben is considered GRAS by reference. Therefore, in this dossier, information about methyl paraben will be limited to a discussion of the level utilized in the formation of FNC.

2.2. Historical and current uses

BAC is commonly used in cosmetics as a foaming, cleansing and bactericidal agent at concentrations up to 5.0% (Elder, 1989). Lower concentrations (typically 0.002 - 0.02%) are used as a preservative for ophthalmic, optic, nasal and/or dermal OTC drug preparations (Elder, 1989; Rowe, *et al.*, 2006). BAC may also be utilized as hard surface cleaner, wood preservative, or pesticide for nursery ornamentals, turf, decorative ponds, or swimming pools (EPA, 2006). Currently, the only food use of BAC is as component of adhesives for food packaging.[9] The related quaternary ammonium salt cetylpyridinium chloride (CPC) is used as an antimicrobial agent to treat the surface of poultry carcasses (Bai *et al.*, 2007).

2.3. Regulatory status of BAC and related substances

The regulatory status of BAC within FDA is summarized in Table 1. BAC is approved for use as a component of adhesives for food packaging[10] and for several non-food uses. BAC (CAS No. 8001-54-5) is approved for use as an ingredient in ophthalmic preparations exempted

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[7] The Code of Federal Regulations (21 CFR §170.3(o)(2) designates this category as "Antimicrobial agents," but recognizes the appropriate application of the term "Preservative."
[8] ppm = parts *per* million.
[9] Title 21 of the US Code of Federal Regulations (CFR), section §175.105.
[10] Title 21 of the US Code of Federal Regulations (CFR), section §175.105, 2010. Approved substance is Alkyl (C10-20) dimethylbenzyl ammonium chloride.

from prescription-dispensing requirements[11] and in dandruff/seborrheic dermatitis/psoriasis drug products, skin protectant drug products (including astringent drug products and insect bite and sting drug products) and external analgesic drug products (including insect bite and sting drug products).[12] BAC is also used as an antimicrobial agent in cosmetics, such as baby products, shampoos, hair conditioners and rinses, face and hand creams and lotions, deodorants, personal cleanliness products, mouthwashes, dentifrices and skin fresheners. In veterinary products, BAC is used as a preservative in proparacaine hydrochloride ophthalmic solutions.[13]

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[11] Title 21 of the US Code of Federal Regulations (CFR), section §310.201, 2010.
[12] Title 21 of the US Code of Federal Regulations (CFR), section §310.545, 2010.
[13] Title 21 of the US Code of Federal Regulations (CFR), section §524.1982, 2010.

Table 1. Regulatory status of BAC (FDA)

Agency	Identification	Permitted functionality	Use limits	Reference
FDA	BAC (Alkyl (C10-20) dimethylbenzyl ammonium chloride)	Component of packaging adhesive	cGMP	21CFR §175.105
FDA	BAC (CAS No. 8001-54-5)	Auricular (otic) preparations	≤ 0.02%	Inactive Ingredient for Approved Drug Products*
		Inhalation solution	≤ 20%	Do.
		Intralesional and intramuscular injectable	≤ 0.02%	Do.
		Nasal spray	≤ 0.11%	Do.
		Ophthalmic suspension and ointment	≤ 0.02%	Do.
		Topical lotion	≤ 0.1%	Do.
		Topical shampoo	≤ 0.2%	Do.
		Topical suspension and drops solution	≤ 0.01%	Do.
		Topical solution	$\leq 7.5 \times 10^{-3}$%	Do.
		Nasal solution	≤ 1.0%	Do.
		Nasal solution, spray	≤ 0.02%	Do.
		Ophthalmic solution	≤ 2.0%	Do.

BAC = Benzalkonium chloride; CAS = Chemical Abstract Service; CFR = Code of Federal Regulations, 2010; Do = Same as above; cGMP = current Good Manufacturing Practice; FDA = US Food and Drug Administration; GRAS = Generally Recognized As Safe; NS = not stated. * http://www.accessdata.fda.gov/scripts/cder/iig/index.cfm; site visited May 28, 2013.

Cetylpyridinium chloride (CPC) is a quaternary ammonium salt similar to BAC that may be safely used in food as an antimicrobial agent[14] to treat the surface of raw poultry carcasses. In this use, the concentration of CPC in a solution applied to poultry carcasses is not to exceed 0.8% (8000 ppm) by weight, followed by a potable water rinse if immersion in chiller water is not part of the production process.[15]

BAC-containing formulations that are added directly to water[16] or used to treat hard nonporous surfaces, wood, plants or turf are regulated as pesticides (EPA, 2006). FNC is not subject to regulation as a pesticide when used in processing plants as a processing aid for lettuce or carrots, as it will not be used on a pest.[17,18] BAC is exempted from requirements of tolerance as

[14] As defined in Title 21 of the US Code of Federal Regulations (CFR), section §170.3(o)(2).
[15] Title 21 of the US Code of Federal Regulations (CFR), section §173.375, 2010.
[16] Swimming pools, decorative ponds/fountains, spas, cooling water towers, oil field drilling muds and packing fluids, small process water systems, humidifiers, and cut flower applications.
[17] Personal correspondence from Dennis Edwards, US Environmental Protection Agency, May 6, 2013.
[18] Title 40 of the US Code of Federal Regulations (CFR), section §152.5.

a pesticide when it is used as an ingredient of an antimicrobial pesticide formulation applied to food contact-surfaces in public eating places, dairy processing equipment, and food-processing equipment and utensils, providing the end-use concentration of all quaternary chemicals in solution does not exceed 200 ppm of active quaternary compound.[19]

2.4. Proposed use or uses

The intended use of FNC is as a processing aid in the processing of lettuce and carrot products during the washing of the carrots and lettuce. FNC will be diluted to a 2% solution for this purpose.

3. MANUFACTURING PROCESS, DESCRIPTION AND SPECIFICATIONS

3.1. Manufacturing Process

FNC is manufactured at ambient temperature by combining food grade glacial acetic acid,[20] food grade methyl paraben[21] and USP grade BAC[22] (50% solution) in precise amounts and in a defined sequence. FNC may be mixed in either high density polyethylene (HDPE) or stainless steel tubs. For a batch with a total volume of 6.3 gallons, methyl paraben (118 g, FCC grade) is added to glacial acetic acid (236 g) and stirred until dissolved. USP-BAC solution (118 g) is then stirred into the mixture, until dissolved. Water from the municipal water supply of Memphis, TN is added until a final volume of 6.3 gallons is reached. Larger batches (up to 300 gallons) may be prepared, by proportionately increasing the amounts of methyl paraben, glacial acetic acid, USP-BAC solution and water used for a 6.3 gallon batch (see above) to the desired volume. The water supply for Memphis, TN meets or exceeds standards set by the Environmental Protection Agency (EPA) for possible contaminants (MLGW, 2010).

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[19] Title 40 of the US Code of Federal Regulations (CFR), section §180.940, 2010.
[20] The glacial acetic acid meets Food Chemical Codex (FCC) specifications (FCC, 2012a) and has a minimum purity of 99.5%, a heavy metal content (as Pb) of $\leq$ 0.5 ppm and an iron content of $\leq$ 0.5 ppm.
[21] The methyl paraben meets Food Chemical Codex (FCC) specifications (FCC, 2012b) and has a minimum purity of 99.0%, a loss on drying of $\leq$ 0.5% and a lead content of $\leq$ 2 ppm.
[22] The BAC solution is USP grade (USP, 2010). It contains 50% BAC (CAS No. 8001-54-5) and may contain up to 10% alcohol. The remainder is water.

The final product, FNC is sold in 55 gallon plastic drums, 275 gallon plastic totes or metal rail cars (with liners) for up to 20,000 gallon quantities. The recommended storage temperature for FNC is room temperature. The concentrated FNC will be diluted 1:50 by the user (for a 2% solution), prior to application to carrots or lettuce in processing facilities. Users will be directed to submerge carrots or lettuce in the 2% FNC solution (as incorporated into the wash solution) for five minutes and change solutions daily or when the lot number changes (whichever is sooner). Users will be directed to divert used 2% FNC to water treatment facilities, as *per* local requirements.

3.2. Description and Specifications

The physical and chemical properties for FNC are provided in Table 2. FNC is a clear, colorless liquid with a slight vinegar odor. FNC is readily soluble in cold and hot water and very soluble in alcohol. The BAC component of FNC is a quaternary salt that has a low vapor pressure (3.53×10^{-12} mm Hg) (EPA, 2006); therefore, it will not volatize under expected storage conditions.

Table 2. Physical and chemical properties of Free N Clear™ Concentrate *

Characteristic	Value
Physical state	Liquid
Color	Colorless
Appearance	Clear
Odor	Slight vinegar
Specific Gravity	0.98 - 1.049
Solubility in water	Complete
pH	3.06 - 3.45
Freezing point	30.2°F
Boiling Point	212°F

*Information obtained from J. Wheeler, Marvel Technologies, personal communication, December 14, 2012.

Permissible concentrations of the components BAC, acetic acid and methyl paraben in FNC are shown in Table 3. Measured concentrations of BAC, methyl paraben and acetic acid in 1:50 dilutions (2% solutions) of lots of FNC meeting specifications (lots 44 and 214) averaged

74 ppm (74 mg/L or 0.0074%), 80.5 ppm (80.5 mg/L or 0.00805%) and 239 ppm (239 mg/L or 0.0239%), respectively.

Table 3. Specifications for Free N Clear™ Concentrate

			Batch Analysis Results ($N = 2^*$)	
Analysis	**Method**	**Specification**	**Range**	**Average**
Benzalkonium chloride (BAC)	Microbac	2700 ± 200 ppm	2638 (Lot 214) - 2777 (Lot 44)	2708
Acetic acid	NREL/TP-510-42623	8200 ± 1000 ppm	7383 (Lot 44) - 9011 (Lot 214)	8197
Methyl paraben	Modified Agilent	2900 ± 215 ppm	2703 (Lot 214) 3029 (Lot 44)	2866

*Lots 44 (prepared 11/2011) and 214 (prepared 11/2012) were analyzed on January 11, 2013 ; ppm = parts *per* million

The methods used to detect BAC, acetic acid and methyl paraben for verification of the components within FNC are as follows:

BAC: Modified USP 34/29 methodology (USP, 2010). The mobile phase for the high performance liquid chromatograph (HPLC) was a 50:50 mixture of methanol: 7.5 mM K_2HPO_4, pH 3 (68:32) and methanol. The flow rate of the mobile phase was 1.0 ml/min, the injection volume was 50 µl, and the UV detector was set to 260 nm. A four point curve from 30 - 600 ppm was used for calibration. A coefficient of determination of 0.9996 was obtained and *percent* recovery was 99.9 - 101.7%, demonstrating that the method accurately detected BAC.

Acetic Acid: NREL/TP-510-42623 (Sluiter *et al.*, 2006). A four point curve from 5 - 100 ppm was used for calibration. A coefficient of determination of 0.9999 was obtained and *percent* recovery was 99.2 - 99.8%, demonstrating that the method accurately detected acetic acid.

Methyl Paraben: Modified Agilent method (Barbas and Ruperez, 2005). The method was modified by use of a larger column (4.6 mm x 250 mm). A six point curve from 10 - 500 ppm was used for calibration. A coefficient of determination of 0.9998 was obtained and *percent* recovery was 100.6 - 101.8%, demonstrating that the method accurately detected methyl paraben.

3.3. Stability

Quaternary ammonium salts such as BAC are known to be chemically stable in aqueous solutions (Fahelelbom, 2013). FNC is stable at room temperature for 12 to 16 months, as evidenced by the following:

- A 2% FNC solution prepared in July, 2010[23] (expected BAC concentration of 100 ppm) that was stored at room temperature contained 96 ppm BAC in April, 2011[24] and 94 ppm BAC in October, 2011.[15]

- A lot of FNC prepared in November, 2011 (lot 44) contained approximately the same amounts of BAC, methyl paraben and acetic acid as a lot prepared in November, 2012 (lot 214) on the analysis date of January 11, 2013 (Table 3).

4. ESTIMATED DAILY INTAKE

4.1. Residue Analyses

4.1.1. Carrots and lettuce

An analysis was conducted by Microbac Laboratories Inc., Wilson, NC to determine the concentration of BAC that would remain on carrots or lettuce after use of 2% FNC as a processing aid.

Two heads of organic, romaine lettuce and a bag of organic baby carrots purchased from Harris Teeter, Wilson, NC were used in the test. FNC (lot 214) was diluted 1:50 with tap water to make approximately 325 ml of a 2% solution. An aliquot of the 2% FNC solution was prepared by filtering an aliquot through a polyvinylidene difluoride (PVDF) filter into an HPLC autosampler vial. The heads of lettuce were placed into two separate gallon-size Ziploc bags. Carrots were allocated into two different gallon-size Ziploc bags (12/bag). The 2% FNC solution (75 ml) was then placed into each one of the bags. Each sample was shaken for approximately 30 seconds and was allowed to sit at room temperature for approximately three hours. A 10-mL

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[23] Production date of Lot No.1, Free N Clear™, according to personal communication from J. Wheeler, Marvel Technologies USA, LLC, dated October 26, 2011.
[24] Personal communication from Charles River Laboratories dated April 16, 2012.

aliquot of solution from each bag was removed with a syringe and filtered through a 0.45 micron PVDF syringe filter (discarding the first mL) into an HPLC autosampler vial. The HPLC method used for detection of BAC was identical to that described under Section 3.2 A coefficient of determination of 1.0 was obtained. Percent recovery was 98.2 - 99.6%. Results of the study are summarized in Table 4. The concentrations of BAC in carrots and lettuce exposed to FNC for three hours were 13.163 and 18.166 ppm, respectively. The residual amounts of BAC remaining on the lettuce and carrots are estimated to be a worst case scenario for use of 2% FNC as a processing aid, as the recommended processing time for treatment is five minutes, rather than three hours.

Table 4. Summary of BAC analyses on carrots and lettuce

Sample	BAC (ppm)	Average BAC (ppm)
BAC 2% solution	75.993	75.993
Carrot (Sample 1)	13.167	13.163
Carrot (Sample 2)	13.160	
Lettuce (Sample 1)	22.109	18.866
Lettuce (Sample 2)	15.624	

BAC = benzalkonium chloride ; ppm = parts *per* million

4.2. Consumption Analyses

The intake profile (amount and frequency) by individuals in USDA's What We Eat in America (WWEIA) Continuing Survey of Food Intakes by Individuals 2007 - 2008 (CDC, 2011) was used to calculate the estimated daily intake (EDI) of BAC for individuals consuming the foods that will be processed with Free N Clear™ *per* this GRAS determination (*i.e.*, carrot or lettuce products).[25] These products are shown in APPENDIX 1. The consumption of BAC from ingestion of the processed lettuce and carrot products was determined using the residual amount of BAC remaining in the lettuce or carrots after treatment with 2% FNC for three hours (18.866 ppm or 13.163 ppm, respectively). The amount of BAC remaining on the produce after treatment is expected to be a worst case scenario, as the recommended exposure time with 2% FNC is five minutes.

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[25] As noted, a 2% solution of Free N Clear™ will be used only on foods for which a standard of identity does not exist.

The means and 90th *percentile* EDIs were calculated for: (1) current intake of BAC from other sources (current); (2) BAC intake following use of 2% FNC on lettuce and carrots and; (3) total estimated EDI from current sources combined with levels from use of 2% FNC on lettuce and carrots. Current daily dietary aggregate exposure to BAC from direct and indirect food contact as well as drinking water exposures for adults is estimated at 0.0066 mg/kg bw/day (EPA, 2006).

The results of the analysis show that the total estimated mean and 90th *percentile* weighted aggregate consumption of BAC from use of a 2% solution of FNC as a processing aid for "eaters only" of carrots and lettuce is 0.025 mg/kg bw/day and 0.053 mg/kg bw[26]/day, respectively (Table 5). The Environmental Protection Agency (EPA's) level of concern for chronic dietary aggregate exposure to BAC is 0.44 mg/kg bw/day (EPA, 2006), which is approximately eight times higher than estimated 90th *percentile* consumption by people who will eat lettuce and carrots processed with a 2% solution of FNC.

Table 5. BAC current intake, predicted intake and total intake (predicted + current) for individuals consuming lettuce and carrots processed with a 2% solution of Free N Clear™

	Per User (mg/kg bw/day)	
BAC intake from:	**Mean**	**90th Percentile**
Current consumption from food and drinking water*	0.0066	0.0132**
Possible maximum consumption with use of 2 % solution of Free N Clear™ on lettuce and carrots	0.0186	0.0398
Total from conventional food (current + added)	0.0252	0.0530

* EPA estimate (EPA, 2006); ** Estimated as two times the mean; BAC = benzalkonium chloride.

5. ABSORPTION, DISTRIBUTION, METABOLISM AND ELIMINATION (ADME)

No information about the absorption, distribution, metabolism and elimination (ADME) of Free N Clear™ was located. However, information about the ADME of BAC is available.

The distribution and disposition of BAC after a single oral dose of a BAC-containing product commercially available in Japan (Osvan®)[27] has been studied in rats (Xue *et al.*, 2004b; Walsh and Fanning, 2008). A dose of 2.5 ml/kg Osvan® (250 mg/kg BAC) was administered to

[26] bw = body weight
[27] Osvan® contains 100 mg/ml BAC. The C12 homologue was the predominant component (89.8%) of the BAC.

30 fasted rats by stomach tube and blood samples were collected by cardiac puncture 1, 2, 4, 8 and 24 hours later (six rats/time point). Rats were terminated and the lung, liver and kidneys were harvested. The authors noted that concentrations of BAC in blood and tissues were substantially higher in animals that aspirated the BAC-containing product, suggesting that BAC is absorbed by the pulmonary blood vessels if inhaled. In animals that did not aspirate BAC, concentrations of BAC in blood and tissues were relatively low (0.01 – 1 μg/g), and did not increase over time, suggesting that only a small amount of orally administered BAC is absorbed through the gastrointestinal tract of rats. Because BAC is a large, positively charged molecule it is poorly absorbed and likely eliminated largely in feces, similar to other quaternary ammonium compounds (Arugonda, 1999).

6. SAFETY EVALUATION

6.1. Free N Clear™ (2% solution)

6.1.1. Acute toxicity

The dose of Free N Clear™ (or a 2% FNC solution) that causes 50% mortality in rats or mice (LD_{50} value) has not been determined. A preliminary dose range finding test for a micronucleus study in rats showed that Sprague Dawley rats to not die or exhibit clinical signs of distress after daily gavage exposure to 20 ml/kg bw of a 2% FNC solution for three days (which delivers a dose of approximately 1.92 mg USP-BAC/kg bw/day[28] (Dolan *et al.*, 2013).

6.1.2. Subchronic toxicity

The subchronic (91-day) oral toxicity of a 2% FNC solution has been tested in a study performed in accordance with the OECD Guideline No. 408 (Dolan *et al.*, 2013). Groups of ten Sprague-Dawley rats/sex were administered 0.5, 1.0 or 5.0 mL/kg bw/day (500, 1000 or 5000 mg/kg bw/day)[29] of the 2% FNC solution by gavage, once daily for 91 days. The test substance was administered as received (1:50 dilution of FNC concentrate in water). A control group of ten rats/sex was administered 5.0 ml/kg bw/day reverse osmosis deionized water by gavage.

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[28] Based on a BAC content of 96 ppm in a 2% FNC solution.
[29] Based on a specific gravity of 1.

All animals survived until study termination and there were no test article-related clinical or ophthalmologic findings. There were no statistically significant differences in food consumption between treated animals or controls at any time. A statistically significant increase in mean body weight of males provided 500 mg/kg bw/day ($p < 0.05$) was observed on Day 36, but at no other time point. Mean body weight of females given 5000 mg/kg bw/day were slightly lower (1 - 5%) than control at most time points; however there were no statistically significant differences between mean body weights of control or treated females (any dose group) at any time point. Mean body weight gains of males given 500 mg/kg bw/day were significantly higher than control ($p < 0.05$) from Days 1 to 8 and mean body weight gains of females exposed to 5000 mg/kg bw/day were significantly lower than control ($p < 0.05$) from Days 8 to 15. Due to their isolated nature, small magnitude, and lack of dose response or similar directional change in both sexes, any differences in body weight or body weight gain between control and treated males or females are not considered to be related to administration of the test material.

There were no toxicologically relevant changes in hematology or coagulation parameters, clinical chemistry or urinalysis. There was an increase in the number of high dose (5000 mg/kg bw/day) males or females exhibiting nitrite-positive urine (a marker for urinary tract infection). Animals that exhibited nitrite-positive urine did not have corresponding increases in numbers of bacteria or white blood cells in urine, suggesting that the nitrite results were falsely positive for bacterial infection. It is possible that the positive nitrite response was due to an end-product of BAC metabolism. The only statistically significant change in any parameter was a decrease in serum aspartate aminotransferase (AST) in females provided 1000 mg/kg bw/day compared to controls. Decreases in liver enzymes are not considered toxicologically relevant and the mean values were well within the range of historical control values for similar-aged rats of the same strain.

There was no toxicological effect of the test material on organ weights, gross pathology or histopathology. The only statistically significant differences in organ weights between treated and control animals were decreased heart/body weight and lung/body weight ratios of males given 500 mg/kg bw/day and increased epididymides/brain weight ratio of males given 5000 mg/kg bw/day (58.6 ± 9.1 in control and 67.2 ± 4.4 in treated). All differences in organ weight

were considered to be spurious or attributed to normal biologic variation given their lack of microscopic correlates and dose-response relationship. Incidences of gross and microscopic findings were similar between groups. One female in the control group showed evidence of gross changes in the abdominal cavity, spleen and pancreatic and mediastinal lymph nodes, and inflammation in the spleen, lung, liver and lymph nodes. The etiology of the pathological findings in this animal was not apparent. All gross and microscopic findings were considered to be incidental or spontaneous background changes of no toxicologic significance.

Results of the study show that the subchronic (91-day) oral no observable adverse effect level (NOAEL) of a 2% FNC solution is 5000 mg/kg bw/day in rats, the highest dose administered. The daily dose of USP-BAC that was delivered to rats exposed to the 5000 mg/kg bw/day dose of 2% FNC solution (the NOAEL), was 0.48 mg BAC/kg bw/day.

6.1.3. Genotoxicity

Free N Clear™ was nonmutagenic in a bacterial reverse mutation (OECD Guideline 471) assay using a plate incorporation (experiment 1) and a preincubation (experiment 2) method (Dolan *et al.*, 2013). Strains used in the study included *Salmonella typhimurium* TA98, TA100, TA1535 and TA1537 and *Escherichia coli* (*E. coli*) WP2uvrA. Each assay was conducted in the presence and absence of metabolic activation with S9 mix prepared from the S9 microsomal fraction of the livers of Aroclor 1254-treated adult, male Fischer rats. The test substance (a 2% solution of FNC) was diluted in saline (0.9%) solution and added to plates within one hour of dilution. Preliminary toxicity tests using *S. typhimurium* TA100 indicated that the maximum concentrations of test material that would not produce excessive cytotoxicity were 100% (undiluted) in the presence of S9 mix and 50% in the absence of S9 mix. Therefore, these concentrations were selected as the highest test concentrations with or without S9 (respectively). Additional concentrations tested in the presence or absence of S9 were 50%, 25%, 12.5%, 6.25%, 3.13% and 1.56%. Saline (0.9%) was used as the vehicle control. The test substance did not induce any significant or dose-dependent increases in the numbers of revertant colonies in any strain tested in the absence or presence of S9 mix. No precipitation was noted in any strain, under any condition. In the plate incorporation experiment (experiment 1), toxicity occurred in

all strains exposed to the 50% concentration in the absence of S9 mix and in strain TA1537 exposed to the 25% concentration in the absence of S9 mix. In the presence of S9 mix, toxicity was observed in stains TA1535, TA1537 and TA100 exposed to 100% test substance. The concentrations that were toxic to cells in experiment 1 were also toxic to the same strains in experiment 2, with additional strains affected by the 25% concentration in the absence of S9 mix. Therefore, concentrations that were tested extended into the toxic range. The test was considered valid because at least two of the vehicle control plates were within historical control values for mean numbers of revertant colonies, at least two of the positive control plates for each strain and activation state exhibited at least 2-fold increases in revertant colonies, and no toxicity or contamination occurred at four or more concentration levels.

The ability of Free N Clear™ to cause clastogenicity was tested in a rat micronucleus test that complied with OECD Guideline No. 474 (Dolan *et al.*, 2013). The test substance was a 2% solution of FNC. Groups of male and female Sprague-Dawley rats were dosed by gavage with 0 mL/kg bw (n = 5/sex), 5 mL/kg bw (n = 5 males), 10 mL/kg bw (n = 5 males) or 20 mL/kg bw (n = 7/sex) test substance or 50 mg/kg cyclophosphamide (n = 3 males) at 0, 24, and 48 hours. All doses of 2% solution of FNC used in the study (5 g/kg, 10 g/kg or 20 g/kg[29]) exceeded the limit dose of 2 g/kg recommended by OECD Guideline No. 474. Further, the gavage volume of 2% FNC solution used for the 20 mL/kg bw dose (2 mL/100 g bw) was the maximum volume recommended by the Guideline. The 20 mL/kg bw dose of the 2% solution of FNC delivered a BAC dose of 1.92 mg BAC/kg bw. The vehicle control was water for irrigation (20 mL/kg). No animal deaths or adverse clinical signs occurred in the any of the dose groups. Animals were euthanized 24 hours after the final dose and bone marrow samples were taken from one femur of each animal. Two-thousand polychromatic erythrocytes (PCE) *per* animal were scored for micronuclei and the frequency of micronucleated PCE (MN-PCE) determined. The numbers of micronucleated normochromatic erythrocytes (MN-NCE) in mature red blood cells were also recorded. The PCE/NCE ratio (a measure of systemic toxicity) was determined by counting a minimum total of 1000 erythrocytes (PCE + NCE) *per* marrow preparation. The test was judged positive if any test group exhibited a greater than 10% increase in MN-PCE over the expected historical control range (0.04 ± 0.05%). There was no indication that any of the doses of test

material (5, 10 or 20 mL/kg/day) increased the frequency of MN-PCE. The highest MN-PCE frequency recorded for the test item was in the high dose group, where an incidence of 0.03% was observed for male or female animals (or both sexes combined). This range is within the expected negative control range for CD rats. There was no indication of bone marrow toxicity in any of the test item dose groups. The assay was considered acceptable as the MN-PCE frequencies for the negative control rats were within the expected historical range and an adequate positive control response was obtained for at least two animals, as well as the positive control dose group as a whole.

6.2. BAC

6.2.1. Acute toxicity

Reported oral LD_{50} values for BAC in mice and rats are 150 mg/kg bw and 234 -300 mg/kg bw, respectively (Alfredson *et al.*, 1951; Rowe, *et al.*, 2006). In a study designed to assess the pharmacokinetics of orally administered BAC, four out of 34 rats receiving 250 mg/kg BAC by gavage died from respiratory toxicity within a 24 hour period (Xue *et al.*, 2004a). Approximately 50% of the rats exhibited symptoms of respiratory toxicity. The authors suspected that the fatalities and respiratory toxicity were due to aspiration of gavaged material. If no aspiration occurred, the rats appeared normal.

In humans, the lethal oral dose ranges from 100 - 400 mg/kg bw (10 - 15% solution). A 70-year old woman who drank a solution containing approximately 200 mg/kg BAC survived after receiving supportive care at a hospital (Van Berkel and de Wolff, 1988). The literature search revealed one case of acute respiratory toxicity in humans after ingestion of a solution containing 10% BAC. Respiratory insufficiency was reported in a two year old girl after ingesting two teaspoons of an antiseptic solution containing 10% BAC (Okan *et al.*, 2007). She developed chemical pneumonitis and upper GI tract bleeding within a week of exposure, but recovered after treatment in an intensive care unit. Therapies included assisted ventilation, enteral feeding, intravenous infusion of a dextrose-electrolyte solution, and treatment with an H_2 receptor antagonist, steroid and antibiotic.

6.2.2. Subchronic and chronic toxicity

The subchronic or chronic oral toxicity of BAC has been examined in a number of studies that were conducted prior to the adoption of Good Laboratory Practice (GLP) guidelines (Table 6). Early studies with BAC of unknown purity indicate variable NOAELs for BAC, depending on concentration, method of administration and/or vehicle. Whereas one two year dietary study in rats showed no adverse effects at up to approximately 125 mg BAC/kg bw/day (0.25% or 2500 ppm in the diet) (Alfredson *et al.*, 1951), another showed decreased growth at approximately 31.5 mg/kg bw/day (0.063% or 630 ppm in the diet) (Fitzhugh and Nelson, 1948). In both studies, increased mortality, decreased weight gain, diarrhea, gastritis and gross and microscopic changes in the stomach and small intestine were noted in rats administered approximately 250 mg/kg bw/day (0.5% in the diet) BAC. Conversely, no overt adverse effects were found in rats ingesting feed containing 3000 mg/kg bw/day BAC for 4 - 5 weeks (Harshbarger, 1942). Although results of these early studies provide some information about the toxicity of BAC, they are not of sufficient quality for risk assessment purposes.

Results of subchronic or chronic toxicity studies in rats, dogs or guinea pigs involving administration of BAC *via* gavage are also somewhat variable, but in general show lower NOAELs than when administered in the diet. In dogs, the 52-week oral (gavage) NOAELs for BAC when diluted in water or milk as the vehicle are < 12.5 mg/kg bw/day and 25 mg/kg bw/day, respectively (Coulston *et al.*, 1961). No overt signs of toxicity are observed in guinea pigs exposed to 25 mg BAC/kg bw/day by gavage (water vehicle) for one year. Whereas one study showed histological changes in the stomach and intestine of rats administered 5-25 mg BAC/kg bw/day for two years by gavage (water vehicle) (Shelanski, 1949), another showed no effects of 50 mg BAC/kg bw/day when administered by gavage (milk or water vehicle) (Coulston *et al.*, 1961).

EPA FIFRA guideline subchronic toxicity studies in rats and mice and chronic toxicity studies in dogs, mice and rats[30] have been conducted on materials described as "ADBAC C12-

[30] The studies were performed according to FIFRA guidelines 82-1, 83-1(b), 83-2, and 83-5, which have been replaced by OPPTS numbers 870.3100, 870.4100, 870.4200 and 870.4300, respectively

16" or "ADBAC C12-18", in support of an EPA high production volume (HPV) test plan for ADBAC published online in 2011 (TRS, 2011b). The test substance was administered by the dietary route in all of the studies. Summaries of these unpublished studies are provided in an appendix to the test plan (Van Miller and Weaver, 1988; Gill *et al.*, 1991; Goldenthal, 1994; TRS, 2011a). These tests apply to USP-BAC (CAS No. 8001-54-5), as it predominantly contains ADBAC C12-16, but also contains 3% ADBAC C18. The results of these studies are similar to those of the published, non-GLP studies conducted from 1948 - 1961 (see paragraph above).

The author of the summaries stated that the NOAELs for subchronic (93 - 96 day) toxicity in rats and mice were 1000 ppm (approximately 70 mg/kg bw/day in rats and 192 mg/kg bw/day in mice). All of the mice and most of the rats did not survive exposure to next highest dose (4000 ppm; approximately 280 mg/kg bw/day in rats and 768 mg/kg bw/day in mice). The summary writer mentioned that clinical signs of toxicity, decreased food consumption and body weights, gross necropsy findings (principally ileus consisting of distended fluid and gas-filled viscera) and histopathologic effects (related to the gastrointestinal changes) were observed in rats exposed to 4000 ppm concentration. In mice exposed to 4000 ppm, clinical signs of toxicity were restricted to the animals that died, and were related to general cachexia and gross necropsy observations of increased amounts of liquid or semisolid material throughout the gastrointestinal tract.

The summary writer also stated that the NOAELs for chronic toxicity in dogs, rats and mice were 14, 50 and 82 mg/kg bw/day, respectively. In rats and mice, the NOAELs for carcinogenicity were the highest doses given (102 and 259 mg/kg bw/day, respectively). Therefore, it was concluded that BAC was not a carcinogen under the conditions of the studies. Responses observed at the lowest observable adverse effect levels (LOAEL) in chronic toxicity/carcinogenicity studies in dogs, rats and mice (35, 102 or 259 mg/kg/day, the highest doses tested) were limited to changes in body weight, food consumption and/or plasma cholesterol.

(http://www.gpo.gov/fdsys/pkg/FR-1998-08-05/pdf/98-20898.pdf). The OPPTS guidelines are available at http://www.epa.gov/ocspp/pubs/frs/publications/Test_Guidelines/series870.htm; sites were visited June 5, 2013.

Altogether, results of guideline studies in dogs, rats and mice are consistent and show that the NOAELs for chronic toxicity (effects on body weight, food consumption and/or plasma cholesterol) are 14, 50 and 82 mg/kg bw/day, respectively. The minimum NOAEL for chronic toxicity (14 mg/kg bw/day) is 264 times higher than the estimated 90th percentile intake of BAC from all sources (0.0530 mg/kg bw/day), including lettuce and lettuce and carrots processed with a 2% solution of FNC. In rats and mice, BAC is not carcinogenic at the highest doses tested (102 and 259 mg/kg bw/day, respectively). The EPA also has concluded that BAC is not carcinogenic (EPA, 2006).

[Remainder of this page is blank]

Table 6. Subchronic and Chronic Oral Toxicity Studies Conducted on BAC

Species/Strain/ Guideline (Number *per* group)*	Dose/Route	Duration	Results/Notes	Reference
White Rat (strain not specified) Non-guideline study	3% (3000 mg/kg bw/day). Dietary administration	28 - 35 days	NOAEL = 3% (300 mg/kg bw/day). No overt adverse effects noted.	Harshbarger (1942)
Osborne-Mendel Rat Non guideline study *N* = 12 males/group	0, 0.063% (63 mg/kg bw/day), 0.125% (250 mg/kg bw/day), 0.25% and 0.5%. Dietary administration	Two years	NOAEL< 0.063 % (63 mg/kg bw/day) LOAEL = 0.063%: Growth suppression 0.25 %: Diarrhea, bloating of the abdomen, brown syrupy material in intestine, distension of cecum, foci of necrosis in GI tract. 0.5%: All rats died within 10 weeks	Fitzhugh and Nelson (1948)
Yale/Sherman/Wistar Rat Non guideline study *N* = 12-13/sex/group	5, 12.5, 25 mg/kg bw/day *via* gavage	Two years	25 mg/kg bw/day: decreased body weight. Increased cell growth in gastric mucosa at unspecified doses	Shelanski (1949)
"Albino" Rat Non guideline study (*N* = 12/sex/group)	0, 0.015%, 0.031%, 0.062%, 0.125%, 0.25% and 0.5%. Dietary administration	Two years	NOAEL = 0.125% (125 mg/kg bw/day) 0.5%: 50% survival. Diarrhea, brown viscid substance in upper GI tract, gastritis, mucosal necrosis of GI tract	Alfredson *et al.* (1951)
SD Rat Non guideline study *N* = 10 males/group	50 or 100 mg/kg bw/day *via* gavage (water or milk vehicle)	12 weeks	50 mg/kg bw/day: "well tolerated" 100 mg/kg bw/day: Reduced weight gain, increased mortality[a]	Coulston *et al.* (1961)
SD Rat EPA FIFRA 82-1 guideline study	100 ppm (7 mg/kg bw/day), 500 ppm (35 mg/kg bw/day) 1000 ppm (70 mg/kg bw/day),	95 - 96 days	NOAEL = 1000 ppm (70 mg/kg bw/day) LOAEL could not be determined due to 100% mortality at 4000 ppm	Van Miller and Weaver (1988)

Species/Strain/ Guideline (Number *per* group)*	Dose/Route	Duration	Results/Notes	Reference
(OPPTS 870.3100) *N* = 10 sex/group, minimum**	4000 and 8000 ppm.[b] Dietary administration		Increased mortality, gross and histological changes in the GI tract at 4000 and 8000 ppm.	
SD Rat EPA FIFRA 83-5 guideline study (OPPTS 870.4300) *N* = 50 sex/group, minimum**	300 ppm (15 mg/kg bw/day), 1000 ppm (50 mg/kg bw/day), 2000 ppm (102 mg/kg bw/day). Dietary administration	Two years	NOAEL (repeated dose toxicity) = 1000 ppm (50 mg/kg bw/day) NOAEL (carcinogenicity) = 2000 ppm (102 mg/kg bw/day) LOAEL (repeated dose toxicity) = 2000 ppm (102 mg/kg bw/day). Reduced body weight and food consumption	Gill *et al.* (1991)
CD-1 Mouse EPA FIFRA 82-1 guideline study (OPPTS 870.3100) *N* = 10 sex/group, minimum**	100 ppm (20 mg/kg bw/day), 500 ppm (94 mg/kg bw/day), 1000 ppm (192 mg/kg bw/day), 4000, 8000 ppm[b]. Dietary administration	93-94 days	NOAEL = 1000 ppm (192 mg/kg bw/day) LOAEL could not be determined due to high mortality at 4000 ppm. Increased mortality, gross and histological changes in the GI tract at 4000 and 8000 ppm.	Van Miller and Weaver (1988)
CD-1 Mouse EPA FIFRA 83-2 guideline study (OPPTS 870.4200) *N* = 50 sex/group, minimum**	100 ppm (16 mg/kg bw/day), 500 ppm (82 mg/kg bw/day) and 1500 ppm (259 mg/kg bw/day). Dietary administration	78 weeks	NOAEL (repeated dose toxicity) = 500 ppm (82 mg/kg bw/day) NOAEL (carcinogenicity) = 1500 ppm (259 mg/kg bw/day) LOAEL (repeated dose toxicity) = 1500 ppm (259 mg/kg bw/day). Reduced body weights and body weight gains	Gill *et al.* (1991)
Guinea pig Non guideline study *N* = 10/sex/group	5, 12.5, 25 mg/kg bw/day *via* gavage	One year	No overt adverse effects or cellular changes observed in major organs (results not further specified)	Shelanski (1949)

Species/Strain/ Guideline (Number *per* group)*	Dose/Route	Duration	Results/Notes	Reference
Mongrel Dog Non guideline study *N* = 1-2/group	0.031, 0.062, 0.12, 0.25, 0.5, 1%. Dietary administration	15 weeks	NOAEL = 0.12%. LOAEL = 0.25%. Decreased body weight at ≥ 0.25%. Moribundity, mortality and gross and histological changes in the GI tract at ≥ 0.5%.	Alfredson *et al.* (1951)
Beagle Dog Non guideline study *N* = 3/group	12.5, 25 and 50 mg/kg bw/day *via* gavage (10% solution in water or milk vehicle)	One year	Water vehicle: NOAEL < 12.5 mg/kg bw/day. Gross and microscopic changes in the intestine at12.5 mg/kg bw/day. Vomiting and increased mortality at higher doses Milk vehicle: NOAEL 25 mg/kg bw/day. Gross changes in the intestine at 50 mg/kg bw/day	Coulston *et al.* (1961)
Beagle Dog EPA FIFRA 83-1(b) guideline study (OPPTS 870.4100) *N* = 4/sex/group	120 ppm (4 mg/kg bw/day), 400 ppm (14 mg/kg bw/day) and 1200 ppm (35 mg/kg bw/day). Dietary administration	One year	NOAEL = 400 ppm (14 mg/kg bw/day) LOAEL = 1200 ppm (35 mg/kg bw/day). Decreased body weight, food consumption and cholesterol	Goldenthal (1994)

EPA = Environmental Protection Agency; FIFRA = Federal Insecticide, Fungicide and Rodenticide Act; GI = gastrointestinal; LOAEL = lowest observed adverse effect level; NOAEL = no observable adverse effect level; ppm = parts *per* million; SD = Sprague-Dawley

*If available from reference; ** According to guideline. Numbers of animals tested were not mentioned in the reference. [a] Unclear if tissue examinations were performed; [b] Due to mortality in the 4000 and 8000 ppm groups, actual doses could not be calculated.

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6.2.3. Genotoxicity

The genotoxicity of BAC has been evaluated in several *in vitro* assays. Results of bacterial mutagenicity studies with BAC are overwhelmingly negative (Elder, 1989), however, chromosome aberration studies are variable. Results of one study show that concentrations of up to 30 μM BAC (approximately 10.8 mg/L) are not clastogenic in a Syrian Hamster Embryo (SHE) cell study (Hikiba *et al.*, 2005). Another study showed that 1 or 3 mg/L BAC caused an equivocal increase in sister chromatid exchanges in SHE cells (Fukuda, 1987). A 1.0 mg/L concentration of BAC increased the frequency of micronuclei in cultured human lymphocytes (Ferk *et al.*, 2007). DNA damage occurs in cultured human respiratory epithelial cells exposed to 0.02% BAC (200 mg/L) (Deutschle *et al.*, 2006). In rats administered 250 mg/kg BAC by the oral route, the concentration of BAC in plasma and tissues is approximately 0.1 – 1 μg/g (mL), which is approximately equal to the lowest concentration of BAC that caused genetic toxicity *in vitro* (1 mg/L). As mentioned in Section 6.1.3 above, a 20 mL/kg dose of 2% FNC (which delivers a BAC dose of 1.92 mg BAC/kg bw) does not increase the frequency of micronuclei in bone marrow of rats. These findings suggest that the plasma and tissue concentrations that could arise from rats exposed to 1.92 mg BAC/kg bw from a 2% solution of FNC (an estimated maximum of 7.7 μg/L)[31] are substantially lower than those that produce genotoxicity *in vitro*. The plasma concentration of BAC in people consuming lettuce and carrots processed with 2% FNC would be even lower than 7.7 μg/L, as the total amount of BAC consumed by these individuals (0.053 mg/kg bw/day) is 36-times lower than the BAC dose associated with this plasma concentration in rats (1.92 mg BAC/kg bw/day). In conclusion, the maximum tissue and plasma concentrations of BAC in people consuming 90th percentile quantities of lettuce and carrots processed with 2% FNC would be substantially lower than the concentrations of BAC that are clastogenic in some *in vitro* assays. Therefore, the positive results of some *in vitro* clastogenicity studies with high concentrations of BAC are not relevant for the safety assessment of BAC in 2% FNC.

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[31] Assumes that absorption is linearly proportional to dose. 250 mg/kg BAC divided by 1.92 equals 130, The plasma concentration of BAC at a dose of 250 mg/kg BAC (1 mg/L) divided by 130 equals 7.7 μg/L.

6.2.4. Reproductive or Developmental Toxicity

The potential for Free N Clear™ to cause reproductive or developmental toxicity has not been tested. Studies conducted prior to adoption of GLP guidelines showed no overt adverse effects of up to 25 mg/kg bw/day BAC on "fertility"[32] of rats or guinea pigs (Shelanski, 1949).

Four studies have been conducted to examine the developmental toxicity of BAC (Table 7). An EPA FIFRA guideline[33] two generation reproductive toxicity study in Sprague-Dawley rats has been conducted on a material described as "ADBAC C12-16" in support of an EPA high production volume (HPV) test plan published online in 2011 (TRS, 2011b). This test applies to USP-BAC (CAS No. 8001-54-5), as it predominantly contains ADBAC C12-16. A summary of the unpublished study is provided in an appendix to the published EPA HPV test plan for ADBAC (Neeper-Bradley, 1992; TRS, 2011a). The results of this study are similar to those of the published, non-GLP study conducted by Shelanski (1949) (see paragraph above). Doses used in the study were 300, 1000 and 2000 ppm in feed (approximately 22, 73 or 145 mg/kg bw/day, respectively). The author of the summary stated that the no observable effect level (NOEL) for toxicity to parental animals or offspring was 1000 ppm (73 mg/kg bw/day). Effects noted at the lowest observable effect level (LOEL) of 2000 ppm (145 mg/kg bw/day) were reduced body weight or reduced body weight gain of parental animals and pups. Reproductive parameters were not affected by treatment with up to 2000 ppm (145 mg/kg bw/day).

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[32] Endpoints measured to assess "fertility" were not described.

[33] The study was performed according to FIFRA guideline 83-4, which has been replaced by OPPTS number 870.3800 (http://www.gpo.gov/fdsys/pkg/FR-1998-08-05/pdf/98-20898.pdf). The OPPTS guideline is available at http://www.epa.gov/ocspp/pubs/frs/publications/Test_Guidelines/series870.htm, sites visited June 6, 2013.

Table 7. Developmental Toxicity of BAC

Species/Strain/ Guideline (Number *per* group)*	Dose/Route	Duration	Results/Notes	Reference
Wistar Rat Study comparable to OECD Guideline 414 *N* = 22 - 37/group	0, 5, 15, 50 g/kg bw/day	Treatment: GD 6-15 Termination: GD 20	NOEL (maternal toxicity): 15 mg/kg bw/day NOEL (developmental toxicity): 50 mg/kg bw/day LOEL (maternal toxicity):50 mg/kg bw/day. Increased mortality.	Knickerbocker and Stevens (1977)
SD Rat EPA FIFRA 83-3 guideline study (OPPTS number 870.3700) *N* = 25/group	0, 10, 30, 100 mg/kg bw/day	Treatment: GD 6-15 Termination; GD 21	NOEL (maternal toxicity): 10 mg/kg bw/day NOEL (developmental toxicity): 100 mg/kg bw/day LOEL (maternal toxicity): 30 mg/kg bw/day. Reduced food consumption, audible respiration. Perioral wetness noted at 100 mg/kg bw/day.	Neeper-Bradley (1992)
ICR/JCL Mouse Non guideline study *N* = 5 - 20/group	0.001, 0.05, 0.1, 3, 10, 30 mg/kg bw BAC	Treatment: GD 0-6 Termination: GD 13 or Treatment: GD 0-17 Termination: GD 17	NOAEL (developmental toxicity): 30 mg/kg bw/day	Momma *et al.* (1987)
NZW Rabbit EPA FIFRA 83-3 guideline study (OPPTS number 870.3700) *N* = 16/group	0, 1, 3 or 9 mg/kg bw/day	Treatment: GD 6-18 Termination; GD 29	NOEL (maternal toxicity): 3 mg/kg bw/day NOEL (developmental toxicity): 9 mg/kg bw/day LOEL (maternal toxicity): 9 mg/kg bw/day. Hypoactivity, labored respiration	Neeper-Bradley (1992)

BAC = benzalkonium chloride; EPA = Environmental Protection Agency; FIFRA = Federal Insecticide, Fungicide and Rodenticide Act; LOAEL = lowest observed adverse effect level; NOEL = no observable adverse effect level; NZW = New Zealand White; SD = Sprague-Dawley; *If available from reference

Doses of 0.001, 0.05, 0.1, 3, 10 and 30 mg/kg bw BAC (Japanese Pharmaceutical Grade) were tested for the ability to cause developmental toxicity in mice in three separate experiments (Momma *et al.*, 1987). In the first experiment, 3, 10 or 30 mg/kg bw/day BAC or vehicle (purified water) was administered by gavage to groups of 9 - 12 pregnant mice from Gestation Days 0 - 6. There was no effect of any dose of BAC on body weight, food consumption or the

general appearance of the dams. No significant differences were observed between the control group and the respective treatment groups for pregnancy rate, implantation number, numbers of dead or resorbed fetuses, numbers of viable fetuses, sex ratio or viable fetus body weight. External abnormalities were not observed in any group. The authors stated that there was a trend for decreased pregnancy rate in the 10 and 30 mg/kg bw/day treated groups and a trend for increased numbers of dead and resorbed fetuses in the 30 mg/kg bw/day treated groups; however, values were not statistically significant from controls. In additional experiments, lower doses of 1, 50 or 100 µg/kg bw/day BAC were administered to mice from Gestation Days 0 - 6 and 1 or 50 µg/kg bw/day BAC was administered Gestation Days 0 - 18 (entire pregnancy period). Significant differences were not observed for any variable measured between treated groups and the control group (Momma *et al.*, 1987). The authors concluded that the results of their experiments suggested that in mice, exposure to the high dose of BAC (30 mg/kg bw/day) caused inhibition of implantation or abortion, but that doses < 100 µg/kg bw/day (the highest dose administered in the second experiment) had no effect on reproductive function. However, in the first experiment, the number of implantations, abortions or any other reproductive toxicity parameter measured in the mice exposed to 30 mg/kg bw/day was not significantly different from control. Based on the data reported in the study, the NOAEL for reproductive toxicity in mice is 30 mg/kg bw/day, the highest dose administered in the first experiment.

EPA FIFRA guideline developmental toxicity studies[34] have been conducted in Sprague-Dawley rats and New Zealand White Rabbits with a material described as "ADBAC C12-16", and a study comparable to OECD Guideline 414 has been conducted in Wistar rats with "ADBAC C12-18". These studies were performed in support of an EPA high production volume (HPV) test plan for ADBAC published online in 2011 (TRS, 2011b). These tests apply to USP-BAC (CAS No. 8001-54-5), as it predominantly contains ADBAC C12-16, but also contains 3% ADBAC C18. Summaries of the studies are provided in an appendix to the test plan (Knickerbocker and Stevens, 1977; Neeper-Bradley, 1992; TRS, 2011a). ADBAC was provided by gavage for all of the studies. NOELs in the studies performed with Wistar or SD rats were 15

[34] The studies were performed according to FIFRA guideline 83-3, which has been replaced by OPPTS number 870.3700 (http://www.gpo.gov/fdsys/pkg/FR-1998-08-05/pdf/98-20898.pdf). The OPPTS guideline is available at http://www.epa.gov/ocspp/pubs/frs/publications/Test_Guidelines/series870.htm, sites visited June 6, 2013.

or 10 mg/kg bw/day for maternal toxicity and 50 or 100 mg/kg bw/day for developmental toxicity (respectively). In SD rats, doses of 30 mg/kg bw/day or 100 mg/kg bw/day from GD 6-15 were associated with reduced food consumption during GD 6-9. However, there was no effect of either of these doses on maternal or fetal body weight or any index of reproductive or developmental toxicity measured in the study. In the study performed in Wistar rats, three dams exposed to 50 mg/kg bw/day died. No other adverse effects of treatment were mentioned in the HPV summary. It is unclear whether the deaths were due to gavage error or treatment with BAC. However, it should be noted that no deaths were reported in SD rats exposed to up to 100 mg/kg bw/day BAC. In New Zealand rabbits NOELs for maternal and developmental toxicity were 3 mg/kg bw/day and 9 mg/kg bw/day (highest dose provided), respectively. Hypoactivity and labored or audible respiration were observed in rabbits exposed to 9 mg/kg bw/day. Based on information provided for these studies, it is reasonable to assign a NOEL for developmental toxicity in rats and rabbits of 100 mg/kg bw/day and 9 mg/kg bw/day BAC, respectively.

6.2.5. Other toxicity studies

BAC has been tested for the ability to cause eye irritation, skin irritation and sensitization. The results of the studies are not critical to the determination of safety of a GRAS ingredient, but help identify whether certain precautions should be taken during handling.

6.2.5.1. Eye irritation

Microscopic changes in the corneal epithelium are observed in eyes of rabbits after ocular exposure to $\geq$ 0.01% (100 ppm) BAC (Bernholc, 1984; Elder, 1989; Furrer *et al.*, 2002). In humans, solutions of 0.03 - 0.04% BAC (300 - 400 ppm) may cause reversible eye irritation (Elder, 1989). A 0.02% (200 ppm) solution of BAC is generally not irritating to human eyes; however, a few cases of conjunctival redness have been reported at this concentration (Elder, 1989). Use of eye protection will be recommended for workers handling FNC concentrate, as the amount of BAC in the concentrate is greater than 200 ppm.

6.2.5.2. Skin irritation

BAC is irritating to human skin at concentrations of 1 - 10%. The maximum concentration of BAC that does not produce irritation to intact skin is 0.1% (1000 ppm).

Concentrations < 0.1% have caused irritation in people with contact dermatitis or broken skin (BIBRA, 1989; Elder, 1989). In an annual review of cosmetic ingredient safety conducted in 2006 and published in 2008, the Cosmetic Ingredient Review (CIR) Expert Panel stated that up to 0.1% BAC is safe for use as a cosmetic ingredient in certain products that are applied to skin or eyes (Andersen, 2008). Use of gloves will be recommended for workers handling FNC concentrate, as the concentration of BAC in the concentrate is greater than 0.1%.

6.2.5.3. Sensitization

Various studies involving repeated dermal or intradermal applications of BAC and challenge with 0.01 - 0.3% (100 - 3000 ppm) solution have shown that BAC can induce sensitization in guinea pigs and mice (BIBRA, 1989). Skin sensitization has been noted in patients tested with BAC concentrations ranging from 0.01 to 0.7% (100 - 7000 ppm). However, in patch tests carried out in the general population and in healthy volunteers, no sensitivity to 0.1% (1000 ppm) BAC was detected (Elder, 1989). It has been suggested that the sensitization response to BAC is not mediated by an immune response, but to the irritant properties of BAC (Uter *et al.*, 2008).

Inhalation of nebulizer solutions containing 250 ppm BAC has been associated with bronchoconstriction in some patients with asthma (Beasley *et al.*, 2001). A study involving 30 subjects with bronchial asthma and ten normal controls inhaling up to three 600 µg nebulized doses of BAC in a jet nebulizer showed that BAC exposure did not cause bronchoconstriction (defined as a ≥ 15% decrease in forced expiratory volume over one second (FEV_1)) in normal subjects, but did in 6/30 (20%) of the asthmatics. The *percentage* decrease in FEV_1 was significantly higher in asthmatics (2.69%, 5.36% and 5.30% after each dose) than normal subjects ($p < 0.05$), and significantly higher in asthmatics with higher bronchial hyper-responsiveness (BHR) than those with lower BHR. BAC-induced bronchoconstriction was reversed with a short-acting β-2 agonist. The study suggest that bronchoconstriction to BAC does not occur in nonasthmatics, but may in asthmatics (particularly in those with high airway sensitivity).

Cases of bronchoconstriction or asthma have been reported in people occupationally exposed to BAC. A statistically significant association has been found between the prevalence of

mild bronchial responsiveness in pig farmers and use of BAC as a disinfectant (Vogelzang *et al.*, 1997). A 22-year old woman working in a factory that manufactured cleaning solutions developed wheezing, shortness of breath and a skin rash, which were precipitated by exposure to a toilet bowl cleaner containing BAC (Bernstein *et al.*, 1994). Three nurses developed symptoms of asthma after handling disinfectant solutions containing BAC (Purohit *et al.*, 2000). Concentrations of BAC in the disinfectant solutions were reported in two of the cases - 10% and 40%. In all cases mentioned above, the diagnosis of BAC-induced bronchoconstriton was confirmed by bronchial challenge tests.

The mechanism by which BAC causes bronchoconstriction is unknown. As intradermal administration of BAC can cause skin sensitization, it has been suggested that the mechanism of BAC-induced bronchoconstriction is mediated by IgE (Lee and Kim, 2007). However, as BAC can elicit non-IgE mediated histamine release from rat mast cells and the BAC-induced bronchoconstriction response can be blocked by antihistamines, the bronchoconstrictor response is likely mediated by a non-immunological response involving release of histamine. A placebo-controlled study in twelve asthmatic subjects showed that use of terfenadine (an H1-receptor antagonist) inhibited the initial, 5-minute bronchoconstrictor response to BAC by 40%, but had a minimal effect on the longer term (45-minute) response (Miszkiel *et al.*, 1988). The authors concluded that the major mechanism by which BAC exerts its adverse effects on the airways of asthmatics does not involve histamine release. However, as terfenadine does not block H2 receptors, it is altogether possible that the effects of BAC on the airways were mediated by an H2-dependent pathway. Other possible mechanisms include BAC-induced release of lipid-derived mediators (*e.g.* prostaglandins) from mast cells into airways or direct stimulation of nerve endings, irritant receptors or bronchial smooth muscle (Miszkiel *et al.*, 1988).

Although skin sensitization and bronchoconstriction in response to BAC exposure are rare and have occurred at concentrations higher than the concentration of BAC in FNC concentrate, use of gloves and respiratory protection will be recommended for workers handling FNC concentrate. These protections will not be required for consumers of lettuce or carrots processed with 2% FNC, as the concentrations of BAC remaining in the foods after processing

are substantially lower than the concentrations of BAC shown to cause skin sensitization or bronchoconstriction.

6.2.6. Other considerations

6.2.6.1. Possible tolerance of *E. coli* to BAC

The concentration of BAC in 2% FNC and the residual levels on carrots and lettuce are highly unlikely to result in increased resistance to either BAC or antibiotics. Reported minimum inhibitory concentrations (MIC) of BAC against *E. coli* are 6.2 ppm[35] to 13 ppm (Bore *et al.*, 2007; Hirayama, 2011; Moen *et al.*, 2012). Exposure of *E. coli* to a subminimal inhibitory concentration (25% below the MIC) of BAC has been shown to select for a small population (approximately 1-5% of the initial population) to survive and regain similar morphology and growth rate as non-exposed cells. This subpopulation maintains tolerance to BAC after serial transfers in medium without BAC. Experiments *in vitro* have shown that *E. coli* grown in medium initially containing 2 ppm BAC and passaged in medium containing incremental concentrations of BAC develop decreased sensitivity to BAC and antibiotics such as chloramphenicol, florfenicol, ciprofloxacin, nalidixic acid, ampicillin and cefoxatime (Bore *et al.*, 2007). The concentration of BAC in a 2% solution of FNC (75 - 96 ppm) is much higher than 2 ppm, suggesting that use of 2% FNC in lettuce and carrot processing plants will not lead to growth of *E. coli* that is tolerant to BAC in the processing plants. Further, residual concentrations of BAC remaining on lettuce and carrots processed with 2% FNC (19 and 13 ppm, respectively) are higher than 1-3 ppm, suggesting that use of 2% FNC as a processing aid will not lead to development of antibiotic-resistant strains of *E. coli* in humans ingesting the treated lettuce or carrots.

6.2.6.2. Potential Environmental Effects

The proposed use of a 2% solution of FNC will not significantly alter the concentration or distribution of BAC which is naturally present in the environment. This conclusion is based on the following:

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[35] Calculated using a reported value of 17.3 μM and a molecular weight of 360.

- Users will be directed to divert used 2% FNC to water treatment facilities, as *per* local requirements;
- BAC has a strong tendency to bind to soil and sediment (EPA, 2006) and will bind to sludge in water treatment plants, removing BAC from water (TRS, 2011b);
- Under appropriate conditions and concentrations, BAC degrades into 60% CO_2 within 13 days and is therefore considered readily biodegradable (EPA, 2006)
- Based on physical properties and results of biodegradability studies BAC is expected to be removed from water before it reaches aquatic ecosystems.

7. EVALUATION

Free N Clear™ (FNC) is a proprietary mixture composed of benzalkonium chloride (BAC), acetic acid, and methyl paraben in an aqueous base. The concentrated form of FNC is prepared by mixing specified amounts of USP benzalkonium chloride (BAC) solution (50% concentration), methyl paraben, acetic acid and water. FNC is stable at room temperature for 12 to 16 months. FNC concentrate is diluted 1:50 (*i.e.*, a 2% FNC solution) for use. Both acetic acid and methyl paraben are safe and suitable ingredients already approved for use in foods; therefore, the safety of BAC as a component of a 2% FNC solution is the primary focus of this dossier. A 2% FNC solution contains 96 - 100 ppm (0.01%) USP-BAC. BAC is currently approved for use as a component of adhesives for food packaging, and for several non-food uses. BAC-containing formulations that are added directly to water or used to treat hard nonporous surfaces, wood, plants or turf are regulated as pesticides. FNC is not subject to regulation as a pesticide when used in processing plants as a processing aid for lettuce or carrots, as it is not being used on a pest.

The intended use of 2% FNC is as a processing aid for lettuce and carrots. Users will be directed to submerge carrots or lettuce in the 2% FNC solution for five minutes and change solutions daily or when the lot number changes (whichever is sooner). Users also will be directed to divert used 2% FNC solution to their water treatment facility before discharge into environmental water to minimize exposure to aquatic organisms.

FNC is nonmutagenic in a bacterial reverse mutation assay in the presence or absence of metabolic activation. In rats, up to 20 mL/kg bw/day of a 2% solution of FNC (maximum dose of 1.92 mg USP-BAC/kg bw/day) does not cause clastogenicity. The NOAEL for 2% FNC in a subchronic (91-day) oral toxicity study in rats is 5000 mg/kg bw/day (the highest dose administered), or 0.48 mg/kg bw/day in terms of BAC content. Guideline studies indicate that the NOAELs for subchronic toxicity of BAC in rats and mice are 70 and 192 mg/kg bw/day, respectively, and for chronic toxicity in dogs, rats and mice are 14, 50 and 82 mg/kg bw/day, respectively. The NOAEL for reproductive toxicity in mice is 30 mg BAC/kg bw/day, and for developmental toxicity in rats and rabbits is 100 mg BAC/kg bw/day and 9 mg BAC/kg bw/day, respectively. In rats and mice, the NOAELs for carcinogenicity were the highest doses given (102 and 259 mg/kg bw/day, respectively). The EPA has concluded that BAC is not a carcinogen. Use of gloves and respiratory protection will be recommended for workers handling Free N Clear™ concentrate, as the amount of BAC in the concentrate could cause eye, skin or respiratory irritation or skin sensitization.

The concentrations of BAC in carrots and lettuce exposed to FNC for three hours are 13.163 and 18.166 ppm, respectively. The residual amounts of BAC remaining on the lettuce and carrots are estimated to be a worst case scenario for use of 2% FNC as a processing aid, as the recommended processing time for treatment is five minutes, rather than three hours. Combining the 90th *percentile* consumption of BAC retained on lettuce and carrots processed with 2% FNC (0.0398 mg/kg bw/day) with the current 90th *percentile* current consumption level of BAC from conventional foods (0.0132 mg/kg bw/day), the estimated 90th *percentile* intake of BAC from conventional foods is 0.0530 mg/kg bw/day. This theoretical intake level represents a conservative estimate because it is unlikely that an individual would consume BAC at its current estimated 90th *percentile* exposure level plus the 90th *percentile* exposure level from lettuce and carrots processed with 2% FNC. Even this conservative estimate, however, is 8 - 9 times less than the EPA's oral concern level of 0.44 mg BAC/kg bw/day and the NOAEL for the 91-day rat study for 2% FNC in terms of BAC (0.48 mg BAC/kg bw/day), 264 times the lowest NOAEL for chronic toxicity of BAC (14 mg/kg bw/day in dogs), 1925 times the lowest NOAEL for carcinogenicity of BAC (102 mg/kg bw/day in rats) and 167 times the lowest NOAEL for reproductive or developmental toxicity of BAC (9 mg/kg bw/day in rabbits).

8. CERTIFICATION

The undersigned authors of this document—a dossier in support of the GRAS determination for the use of benzalkonium chloride (BAC) as a component of Free N Clear™ (FNC), when diluted to a 2% solution for use as a processing aid for lettuce and carrots—hereby certify that, to the best of their knowledge and belief, this document is a complete and balanced representation of all available information, favorable as well as unfavorable, known by the authors to be relevant to evaluation of the substance described herein.

(b) (6)

6 August 2013

Laurie C. Dolan, PhD.
Senior Toxicologist, Burdock Group

Date

(b) (6)

6 August 2013

George A. Burdock, Ph.D.
Diplomate, American Board of Toxicology
Fellow, American College of Nutrition
President, Burdock Group

Date

9. CONCLUSION

Following a critical evaluation of the information available, the Expert Panel has determined that, based on common knowledge throughout the scientific community knowledgeable about the safety of substances directly or indirectly added to food, there is reasonable certainty that BAC is safe under the intended conditions of use, and is therefore Generally Recognized As Safe (GRAS), by scientific procedures, when used as an ingredient of Free N Clear™ (FNC), when a 2% solution of FNC is used as a processing aid on lettuce and carrots.

It is our opinion that other experts qualified by scientific training and experience to evaluate the safety of food and food ingredients would concur with these conclusions.

10. SIGNATURES

(b) (6)

8/9/13

Douglas L. Archer, Ph.D.
Associate Dean for Research, UF/IFAS
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(b) (6)

8/15/13

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12. APPENDIX I

Foods selected for the addition of benzalkonium chloride as a component of Free N Clear™

Food item description	Concentration (ppm)
CARROTS, RAW	13.163
CARROTS, RAW, SALAD W/ APPLES	13.163
CARROTS, COOKED, NS AS TO FORM, NS FAT ADDED	13.163
CARROTS, COOKED, FROM FRESH, NS FAT ADDED	13.163
CARROTS, COOKED, FROM FROZEN, NS FAT ADDED	13.163
CARROTS, COOKED, FROM CANNED, NS FAT ADDED	13.163
CARROTS, COOKED, NS AS TO FORM, FAT NOT ADDED	13.163
CARROTS, COOKED, FROM FRESH, FAT NOT ADDED	13.163
CARROTS, COOKED, FROM FROZEN, FAT NOT ADDED	13.163
CARROTS, COOKED, FROM CANNED, FAT NOT ADDED	13.163
CARROTS, COOKED, NS AS TO FORM, FAT ADDED	13.163
CARROTS, COOKED, FROM FRESH, FAT ADDED	13.163
CARROTS, COOKED, FROM FROZEN, FAT ADDED	13.163
CARROTS, COOKED, FROM CANNED, FAT ADDED	13.163
CARROTS, COOKED, NS AS TO FORM, CREAMED	13.163
CARROTS, COOKED, FROM FRESH, CREAMED	13.163
CARROTS, COOKED, FROM FROZEN, CREAMED	13.163
CARROTS, COOKED, FROM CANNED, CREAMED	13.163
CARROTS, COOKED, NS AS TO FORM, GLAZED	13.163
CARROTS, COOKED, FROM FRESH, GLAZED	13.163
CARROTS, COOKED, FROM FROZEN, GLAZED	13.163
CARROTS, COOKED, FROM CANNED, GLAZED	13.163
CARROTS, COOKED, NS AS TO FORM, W/ CHEESE SAUCE	13.163
CARROTS, COOKED, FROM FRESH, W/ CHEESE SAUCE	13.163
CARROTS, COOKED, FROM FROZEN, W/ CHEESE SAUCE	13.163
CARROTS, COOKED, FROM CANNED, W/ CHEESE SAUCE	13.163
CARROTS, CANNED, LOW SODIUM, NS AS TO ADDED FAT	13.163
CARROTS, CANNED, LOW SODIUM, NO FAT ADDED	13.163
CARROTS, CANNED, LOW SODIUM, FAT ADDED	13.163
CARROT JUICE	13.163
CARROT CHIPS, DRIED	13.163
CAESAR SALAD (W/ ROMAINE)	18.866
ENDIVE, CHICORY, ESCAROLE OR ROMAINE LETTUCE, RAW	18.866
LETTUCE, RAW (INCLUDE LETTUCE, NFS)	18.866
LETTUCE, BOSTON, RAW	18.866
LETTUCE, MANOA	18.866
MIXED SALAD GREENS, RAW	18.866
LETTUCE, COOKED, FAT NOT ADDED IN COOKING	18.866

NFS = Not Further Specified- This indicates that the person in the survey did not state the type of product; ppm = parts *per* million.

Pages 000086-000106 of Curriculum Vitae removed in accordance with the Privacy Act of 1974.

SUBMISSION END