Report of the Scientific Committee of the Spanish Agency for Consumer Affairs, Food Safety and Nutrition (AECOSAN) in relation to the use of an antimicrobial aqueous solution containing hydrogen peroxide, acetic acid and peroxyacetic acid (23/17/15) as a processing aid on citrus fruits and tomatoes, and their wash water

Section of Food Safety and Nutrition

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Reference number: AECOSAN-2016-002

Report approved by the Section of Food Safety and Nutrition of the Scientific Committee on plenary session May, 18th 2016

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Abstract

The company Productos Citrosol S.A. has requested a safety assessment on the use of an aqueous solution containing hydrogen peroxide (23 %), acetic acid (17 %) and peracetic acid (15 %) as a processing aid. As stabilisers, 1-hydroxyethylidene-1,1-diphosphonic acid (HEDP) (<0.2 %) and dipicolinic acid (DPA) (≤0.01 %) are included in the solution.

The proposed use of the processing aid is for the bacterial disinfection of citrus fruits and tomatoes on their arrival at processing plants, and the bacterial disinfection of the water used to wash them. By disinfecting the washing water, it can be employed for the consecutive washing of fruits and vegetables through a recirculation system, keeping the water in suitable conditions and reducing water consumption. The requested dose of use is 0.4 % for citrus fruits and 0.2 % for tomatoes.

In 2013, the Scientific Committee assessed a product of similar composition. On this occasion, the applicant analysed the residues of dipicolinic acid in treatment liquids and in citrus fruits and tomatoes following treatment. From that data, taking into account the least favourable scenario and the consumption of citrus fruits and tomatoes in Europe, estimated daily intake (EDI) was established for the consumption of citrus fruit and tomatoes treated with the processing aid; the risk to the consumer was also assessed using a “margin of safety” (MOS) calculation.

The Scientific Committee concludes that, based on the information provided by the applicant and taking into account the proposed composition and conditions of use, the use of the processing aid does not involve a health risk for the consumer.

Key words

Citrus fruits, tomatoes, processing aid, bacteriological disinfection.

Translated from the original published in the journal: Revista del Comité Científico de la AECOSAN, 23, pp: 21-43

1. Introduction

The company Productos Citrosol S.A., located in Potríes (Valencia), has applied for a safety assessment on the use of an aqueous solution of hydrogen peroxide (23 %), acetic acid (17 %) and peracetic acid (15 %) as a processing aid in the bacterial disinfection of citrus fruits and tomatoes when they reach processing plants, as well as washing water. The processing aid, manufactured by the company Solvay Chemicals International S.A. (Brussels, Belgium) is composed of two active compounds: hydrogen peroxide and acetic acid in an aqueous solution, which form a third active compound, peracetic acid, by chemical equilibrium. To maintain this equilibrium, the stabilisers 1-hydroxyethane-1,1-diphosphonic acid (HEDP) (<0.2 %) and dipicolinic acid (DPA) (≤0.01 %) are used. This is an aqueous solution with similar components to another solution previously assessed by the Scientific Committee of the AECOSAN (2013).

In response to the application, the Management Board of the Spanish Agency for Consumer Affairs, Food Safety and Nutrition (AECOSAN) has asked the Section of Food Safety and Nutrition of the Scientific Committee for a safety assessment of the use of said aqueous solution of hydrogen peroxide, acetic acid and peracetic acid, as a processing aid in the bacterial disinfection of citrus fruits and tomatoes and the washing water of these, considering the "Guidelines indicating the necessary documentation for the assessment of processing aids intended for use in human food" (AECOSAN, 2010).

With regard to hydrogen peroxide, this is authorised in Spain as a decontaminant in water intended for human consumption (BOE, 2003) and an Acceptable Daily Intake (ADI) has not been established by the JECFA (Joint FAO/WHO Expert Committee on Food Additives) (JECFA, 2004a). Acetic acid is a food additive (E260) authorised in the European Union and peracetic acid (PAA) is authorised in human food (as a food additive or processing aid) in countries such as Canada and Australia. It also lacks an established ADI (JECFA, 2004a).

With regard to the stabilisers used in the formula, an ADI has not been established for the individual substances either, although in the case of HEDP, an ADI has been defined when it forms part of antimicrobial peroxyacid solutions (JECFA, 2004a).

As it is not possible to dismiss the presence of detectable residues in the final product (citrus fruit and tomatoes) after the use of this aqueous solution, in accordance with the criteria established in the aforementioned Guidelines, the processing aid is classified as in a situation 4: substance authorised in human food for which the ADI has not been established and the use of which results in the technically unavoidable presence of residues. In line with this situation, the applicant for the product presents information relating to the following aspects:

- Administrative data and general outline.
- Physicochemical characteristics.
- Technological function.
- Analysis of residues: analytical method and validation of the method.
- Studies and data on safety: Level A.
- Study on consumption and evaluation of the expected level of consumer intake.

2. Administrative data and general outline

2.1 Commercial name and composition

The product proposed as a processing aid, which has the commercial denomination Citrocide Plus, is an aqueous solution of hydrogen peroxide (23 %) and acetic acid (17 %) which maintains chemical equilibrium with peracetic acid (15 %) and water. To maintain this equilibrium, two stabilisers [(1-hydroxyethane-1,1-diphosphonic acid (<0.2 %) and dipicolinic acid (≤0.01 %)] are used.

2.2 Intended use of the substance

As a processing aid in the bacterial disinfection of citrus fruits and tomatoes when they reach processing plants, as well as washing water.

2.3 Authorised use in human food

Among the main situations in which use in human food is authorised are:

- Hydrogen peroxide. Authorised in Spain as decontaminant of water intended for human consumption (Royal Decree 140/2003) (BOE, 2003).
- Acetic acid. Food additive (E260) authorised by Regulation (EC) No 1333/2008 (EU, 2008), with a specific maximum dose of *quantum satis.*
- Peracetic acid. Authorised in human food (as a food additive or processing aid) in countries such as Canada or Australia. Solutions containing peracetic acid are also authorised in human food (France and the United States).
- 1-hydroxyethylidene-1,1-diphosphonic acid (HEDP). Authorised in Spain for treating water intended for human consumption. In addition, it is authorised in the United States for the washing process or as an aid when peeling fruit and vegetables and as a disinfectant for meat carcasses. It is also included in the Effective Food Contact Substance (FCS) Notifications database of the Food and Drug Administration (FDA). In Australia, it is authorised as a processing aid in water and as a chelating agent in disinfectants used with meat, fruit and vegetables.
- Dipicolinic acid (DPA) is included in the Effective Food Contact Substance (FCS) Notifications database of the Food and Drug Administration (FDA).

Table 1. List of authorised uses and assessments

Substance	Authorised use/assessment	Country/Reference
Hydrogen peroxide	Regulation (EC) No 853/2004 establishes for gelatines and collagen a hydrogen peroxide residue of 10 mg/kg	European Union (EU, 2004)
	Its use is authorised as a substance for decontaminating water intended for human consumption	Spain (BOE, 2003)
	Favourable toxicological assessment as a processing aid in the processing of blood derivatives and cephalopods	Spain (AECOSAN, 2011)
	Its use is authorised as a processing aid for casings	France (Arrêté, 2006)
	Authorised for use in the production of beer as a clarifying agent (maximum quantity 135 mg/kg), in whey for the maintenance of pH (100 mg/kg) and with oat hulls as a bleaching agent (GMP)	Canada (DJC, 2016)
	Classified as GRAS (Generally Recognized As Safe) (21 CFR 184.1366), used in milk (0.05 %), whey (0.04 %), whey cheese coloured with annatto (0.05 %), starch (0.15 %), corn syrup (0.15 %), dehydrated eggs, stomachs, legs of beef, herrings, wine, tea, wine vinegar and emulsifiers (1.25 %)	United States (FDA, 2016a)
	The additive that is a mixture of peracetic acid, octanoic acid, acetic acid, hydrogen peroxide and HEDP is authorised as a disinfectant for bird carcasses, parts, casings and organs with a maximum peroxyacid concentration of 220 mg/kg for peracetic acid, 110 mg/kg for hydrogen peroxide and 13 mg/kg for HEDP	United States (FDA, 2016c)
	Its use is authorised as a processing aid (bleaching agent) in foods, for which a maximum residue level of 5 mg/kg is laid down	Australia (ANZFSC, 2016)
Acetic acid	Authorised as a food additive (E260) by Regulation (EC) No 1333/2008, with a specific maximum dose of *quantum satis*	European Union (EU, 2008)
Peracetic acid	Authorized for use as a processing agent of peracetic acid in solution with hydrogen peroxide and acetic acid, with egg shells intended for the manufacture of *îlle flotant* (solution at 2.5 % with 4.5 % of peracetic); with peas and green beans intended for sterilisation (500 mg/l of peracetic acid); in starch, corn starch and derivatives (1 kg/tonne); ready-to-eat raw salads (4th range); with blanched spinach intended for freezing (75 mg/l of peracetic) and with wheat before grinding (3 l of solution of 15 % peracetic and 23 % hydrogen peroxide per tonne of wheat)	France (Arrêté, 2006)
	Authorised for the process of washing or help in peeling fruits and vegetables that are not raw, unprocessed materials without exceeding 80 mg/kg in the washing water	United States (FDA, 2016b)

Table 1. List of authorised uses and assessments

Substance	Authorised use/assessment	Country/Reference
Peracetic acid	The additive that is a mixture of peracetic acid, octanoic acid, acetic acid, hydrogen peroxide and HEDP is authorised as a disinfectant for bird carcasses, parts, casings and organs with a maximum peroxyacid concentration of 220 mg/kg for peracetic acid, 110 mg/kg for hydrogen peroxide and 13 mg/kg for HEDP	United States (FDA, 2016c)
	Included in the Effective Food Contact Substance (FCS) Notifications database, essentially forming part of aqueous solutions with acetic acid and hydrogen peroxide	United States (FDA, 2016d)
	Authorised as a food additive (starch modifying agent)	Canada (DJC, 2016)
	Authorised as a processing aid as a bleaching agent for washing and peeling, and as a catalyst with a maximum permitted level of 0.7 mg/kg	Australia (ANZFSC, 2016)
1-hydroxy ethane-1,1-diphoso-phonic (HEDP) acid	Authorised for use as a substance for decontaminating water intended for human consumption (the product must not be present in the water above the detection limit of the best available analysis technique)	Spain (BOE, 2003)
	Its use is authorised as a processing aid for sugar	France (Arrêté, 2006)
	Authorised for the process of washing or help in peeling fruits and vegetables that are not raw, unprocessed materials without exceeding 4.8 mg/kg in the washing water	United States (FDA, 2016b)
	The additive that is a mixture of peracetic acid, octanoic acid, acetic acid, hydrogen peroxide and HEDP is authorised as a disinfectant for meat carcasses, parts, casings and organs with a maximum peroxyacid concentration of 220 mg/kg for peracetic acid, 110 mg/kg for hydrogen peroxide and 13 mg/kg for HEDP	United States (FDA, 2016c)
	Included in the Effective Food Contact Substance (FCS) Notifications database essentially forming part of aqueous solutions with acetic acid, peracetic acid, hydrogen peroxide and dipicolinic acid	United States (FDA, 2016d)
	Authorised as a processing aid in water and as a chelating agent in disinfectants used with meat, fruit and vegetables	Australia (ANZFSC, 2016)
Dipicolinic acid (DPA)	Included in the Effective Food Contact Substance (FCS) Notifications database essentially forming part of aqueous solutions with acetic acid, peracetic acid, hydrogen peroxide and HEDP	United States (FDA, 2016d)
	Present in the spores of bacteria belonging to the *Bacillus* genus and found in large quantities in a traditional Japanese fermented food known as *natto*, a food made from fermented soybeans[1]	Japan

[1]The mean daily intake of dipicolinic acid (DPA) of the Japanese population resulting from the food *natto* is 0.6-4 mg (Ohsugi and Sumi, 2011). In addition, the DPA shows antimicrobial activity against *E. coli*, yeasts and *Vibrio* (Sumi and Ohsugi, 1999) and is being studied for its pharmacological activity in blood circulation (Ohsugi et al., 2005). Some species of the *Bacillus* genus are classified by the EFSA among the microorganisms intentionally added to food as safe (QPS) with the qualification of absence of toxigenic activity and acquired antibiotic resistant genes (EFSA, 2015).

The applicant states that both the hydrogen peroxide and the peracetic acid are notified in Regulation (EC) No 1451/2007 on the second phase of the 10-year work programme referred to in Article 16, section 2, of Directive 98/8/EC of the European Parliament and of the Council concerning the placing of biocidal products on the market, as active substances for the manufacture of said products (EU, 2007). This notification indicates that during a period of transition they can be used whilst these uses are assessed.

In addition, the use of hydrogen peroxide is authorised as an active substance in biocides of product types 1, 2, 3, 4, 5 and 6 (UE, 2015).

2.4 Acceptable Daily Intakes

None of the components of the product has an established ADI.

It is also pointed out that this type of formula has been assessed by several international bodies. Thus, for antimicrobial peroxyacid solutions such as hydrogen peroxide, acetic acid and peracetic acid, including hydroxyethylene diphosphonic acid as a stabiliser, JECFA (2004a) considers the quantities of residues in treated foods not to be any cause for concern in terms of health safety at the moment of their consumption in the intended conditions of use.

3. Physicochemical characteristics

3.1 Composition and detailed formula

The product proposed as a processing aid is an aqueous solution of hydrogen peroxide (23 %) and acetic acid (17 %) in chemical equilibrium with peracetic acid (15 %) and water. As indicated in the application, together with the active compounds, the product contains two stabilisers (1-hydroxyethane-1,1-diphosphonic acid (<0.2 %) and dipicolinic acid (≤0.01 %)). Table 2 shows the detailed formulation of the processing aid.

Table 2. Composition of the processing aid, Citrocide Plus

Component	Function	CAS No	Molecular weight (g/mol)
Hydrogen peroxide	Active substance	7722-84-1	34
Acetic acid	Active substance	64-19-7	60.1
Peracetic acid	Active substance	79-21-0	76.1
1-hydroxyethane-1,1-diphosophonic (HEDP) acid	Stabiliser	2809-21-4	205.02
Dipicolinic acid (DPA)	Stabiliser	499-83-2	167.12
pH= 0.46			

3.2 Product specifications

Table 3 presents the specifications and the technical specification sheet of the product as well as the results of the analyses of three batches of the proposed processing aid.

Table 3. Specifications, technical specification sheet and test results for Citrocide Plus

Component	Specification (% w/w)	Technical sheet (% w/w)	Certificate of analysis (% w/w)		
Hydrogen peroxide	21-24	23	22.5	22.3	22.5
Acetic acid	-	17	-	-	-
Peracetic acid	14.5-15.5	15	15	15.1	14.9
1-hydroxyethane-1,1-diphosophonic (HEDP) acid	<0.2 %	-	0.164±0.010	0.172±0.010	0.179±0.012
Dipicolinic acid (DPA)	≤0.01 %	-	0.00336±0.00018	0.00180±0.00008	0.00974±0.00018

3.2.1 Product stability

The applicant has provided data regarding the stability of the formula with the commercial name Proxitane 15 (with the same composition) to demonstrate that it is stable at ambient temperature, and the loss in concentration of peracetic acid is approximately 1 % w/w per year.

3.2.2 Reactivity

The reactions that take place in the water are those involving the decomposition of the compounds with peroxide groups to form acetic acid and water (EFSA, 2005):

$$H_2O_2 + 2H^+ + 2e^- \longrightarrow 2\,H_2O$$
$$CH_3COOOH + 2H^+ + 2e^- \longrightarrow CH_3COOH + H_2O$$

The reactions that occur in the contact environment are as follows (JECFA, 2004b):

$$H_2O_2 \longrightarrow H_2O + ½\,O_2$$
$$CH_3COOOH + H_2O \rightleftharpoons CH_3COOH + H_2O_2$$
$$CH_3COOOH \longrightarrow CH_3COOH + ½\,O_2$$

JECFA, upon assessing disinfectant solutions containing hydrogen peroxide, peracetic acid, octanoic acid, peroxyoctanoic acid and hydroxyethylene diphosphonic acid, indicates that, in contact with foods, the active ingredients decompose rapidly into non-toxic substances and that the quantities of acetic acid that may remain as a result of the decomposition of peracetic acid do not pose any safety threats. It is also worth highlighting that hydrogen peroxide decomposes rapidly in contact with foods, giving water and oxygen (JECFA, 2004c).

Furthermore, the use of this kind of solution does not seem to negatively affect the nutrient content (vitamin C and β-carotene) present in fruits and vegetables as indicated by JECFA (2006). Similarly, EFSA (2005), in assessing the use of peroxyacids to treat chicken carcasses, concludes that there were no perceived effects on proteins and lipids in the products treated.

4. Technological function

4.1 Alleged technological use

The applicant relates that the alleged technological use is as a bacterial disinfectant of citrus fruits and tomatoes as well as washing waters. Citrus fruits and tomatoes are washed when they arrive at processing plants in order to minimise contamination or recontamination during this first phase of the process. In addition, it helps to reduce water consumption at fruit and vegetable packing plants and prevents the emission of discharges with a high contaminating chemical load into the aquifers, unlike other disinfection methods.

Other benefits indicated by the applicant, in addition to its efficiency and that it does not alter the quality and nutritional value of the foods treated, include the low phytotoxicity and the possibility of its application with fungicides without reducing their effectiveness.

4.2 Target foods or food group

The target foods or groups of foods are citrus fruits and tomatoes.

4.3 Level of use required

The applicant states that the dose of processing aid to be used is 0.4 % for citrus fruits and 0.2 % for tomatoes.

4.4 Justification of use, interest and effectiveness

In accordance with the report from the Scientific Committee dated 2013 (AECOSAN, 2013) and with various studies (FAO/WHO, 2009) (EFSA, 2013), the main pathogenic microorganisms linked to diseases that affect human beings as a result of the consumption of fresh, non-animal products, among which fruit and vegetables, are *E. coli, Salmonella* spp., *Shigella* spp., *Listeria monocytogenes, Cryptosporidium* spp., *Cyclospora* spp. and *Clostridium botulinum.* These sources also cite several enteric viruses as pathogens (Norovirus or the hepatitis A virus).

In fresh fruit and vegetables the largest proportion of contamination from microorganisms pathogenic for human health are due ultimately to factors existing before harvest. Potential sources of contamination at different stages of cultivation can be: irrigation water, fertilisers and manure, contaminated instruments, poor hygiene of field workers, etc. Furthermore, even in fruits and vegetables consumed with minimal post-harvest handling/processing, there is a higher risk of microbiological contamination from cross-contamination in phases subsequent to harvesting, which increases the risk to human health. Processes such as washing and packing are very common practices in the majority of fruits and vegetables intended to be consumed fresh. These processes are crucial aspects when it comes to microbiological contamination.

The processes of post-harvest washing with drinkable water can succeed in eliminating only some of the microorganisms present on the surface of the fruit; they do not act as disinfecting treatment. The post-harvest washing stage is crucial. These processes require the use of large quantities of water, making it essential to recycle water to save resources and minimise the environmental impact of this practice. In fruit and vegetable washing systems with water recirculation, if the water is not disinfected properly, it acts as a means of transferring microorganisms, leading to cross-contamination of the fruit washed.

In the fruit and vegetable sector, the first post-harvest treatment undertaken on vegetable products is washing, which can take place either by immersion in a water tank or using a system known as drencher or pallet shower which enables the fruits or vegetables to be wet to the perfect level. In both methods, proper maintenance of the washing liquid or water is fundamental, because this is recirculated through the fruit pallet by pallet, which sends both the remains of the chemical treatments previously applied to cultivation and some of the dirt from the harvest (leaves, branches, earth, etc.) and from the fruit itself, as well as pathogenic microorganisms and spores deposited in vegetable matter. This situation causes the accumulation of contamination to increase considerably with each recirculation, making the device a source of dissemination of microorganisms which can affect the safety of the products. To avoid the washing water becoming a channel through which infection is spread by cross-contamination, it must be ensured that its microbiological quality is maintained. For this, disinfecting products can be used, always making sure that degradation products and residues from the antimicrobial agent used do not pose a risk to the health of the consumer or the environment, that they do not alter the organoleptic properties of the fruit or vegetable (Gil et al., 2009) (Kyanko et al., 2010) and that they can be combined with plant protection products without degrading them.

As regards the effectiveness of the technological processing aid proposed, it is stated that the behaviour of these hydrogen peroxide, acetic acid and peracetic acid solutions is similar to that of chlorogenic acids. In other words, this kind of solution has high oxidising power but, unlike chlorogenic acids, their action is less corrosive, they have a wider range of action, they are effective in the presence of organic material and hard water and the products they generate when they react are oxygen, water and acetic acid. Another major advantage when compared with chlorination (the most frequently used disinfection method in the sector) is the elimination of the hazard posed by the formation of trihalomethanes and chlorine vapours, as the oxidation reaction of the organic material resulting from the action of the peracetic acid generates oxygen and acetic acid, substances which are not toxic (Vero et al., 2004) (Gil et al., 2009).

The applicant states that the mixtures of peracetic acid/hydrogen peroxide belong to a type of disinfectant with one of the broadest spectrums of activity, high efficiency in varied ranges of pH and operating temperatures, and the presence of organic matter is not a limiting factor. In addition, it is highlighted that after washing a certain quantity of fruit and vegetables, the washing water must be disposed of, implying a significant waste of potable water when it is not recovered and considerable discharge.

It is also noted that the processing aid is compatible with the authorised plant-protection active substances, with no signs of degradation when the mixes are disinfected with this product. In this respect, the applicant has submitted the results of a compatibility study for Citrocide Plus with authorised fungicides, concluding that they are compatible.

4.4.1 Studies on effectiveness

In order to establish a minimum dose of use necessary to ensure an effective disinfection in all the requested uses without causing phytotoxicity or variations in the organoleptic properties of the citrus fruit and tomatoes, the applicant has submitted the results of the following tests:

- Establishment of the minimum doses of the processing aid, Citrocide Plus, effective for the bacterial disinfection of citrus fruit, tomatoes and their treatment mixes.
- Assessment of the effectiveness on the surface of the plant and vegetable products and in their treatment mixes against *Enterococcus faecalis, Escherichia coli* and *Clostridium perfringens*.
- Control of the microbiological contamination on the surface of fruit and vegetable products and their treatment mixes at the minimum corresponding doses during the period of use.
- Study of the phytotoxic effects on citrus fruit and tomatoes.

4.4.1.1 Establishment of minimum effective doses of processing aid

Two pilot tests were conducted at industrial plants to establish the minimum dose required to maintain the treatment mix recirculating in adequate microbiological conditions. Increasing doses of the processing aid, Citrocide Plus, were tested and their effectiveness against bacteria tested.

For tomatoes, doses of the processing aid of between 0.03 and 0.23 % were tested on the post-harvest treatment mix, establishing as the conventional wash made using potable water as the control.

For citric fruits (oranges and clementines) doses of between 0.10 and 0.60 % were tested. For lemons, given the results obtained for oranges, two doses were tested in order to confirm the results obtained.

The results obtained showed that to guarantee the bacteriological hygiene of the treatment mix on tomatoes at all times, a minimum dose of 0.20 % of the processing aid is required. In the case of citrus fruits, the minimum necessary dose required to control bacterial contamination in the treatment mixes was found to be 0.40 %.

4.4.1.2 Effectiveness against *Enterococcus faecalis, Escherichia coli* and *Clostridium perfringens*

The applicant company has provided the results of a trial conducted in an independent laboratory. During this trial the microbiological parameters were collected as established in Royal Decree 140/2003 laying down the health criteria for the quality of water intended for human consumption (*E. coli, Enterococcus, Clostridium perfringens*) (BOE, 2003).

ATTC strains of *Escherichia coli, Enterococcus faecalis* and *Clostridium perfringens* were inoculated in samples of oranges, tomatoes and water at two contamination levels: between 10 and 100 CFU and at around 100 CFU. Next, the samples of oranges and tomatoes were immersed in a suspension of the processing aid, Citrocide Plus in water (at 0.4 % in the case of oranges and at 0.2 % in the case of tomatoes) for 30 seconds, left to dry and the subsequent count was made.

In the case of water, the sample was inoculated and the processing aid was added at 0.2 and 0.4 %, the subsequent count was made and various contact times (30 seconds, 1 minute, 5 minutes and 15 minutes) considered.

In all the cases, the recovery percentage after inoculation was also observed by analysing control samples (not treated with the processing aid).

To determine aerobic bacteria, the Plate Count Agar culture medium was used (incubated at 30 ºC for 72 hours). For *Clostridium perfringens* the culture medium used was Perfringens Agar Base incubated to 37 ºC for 48 hours.

The results obtained showed inhibition efficiencies of 98-100 % in oranges and 95-100 % in tomatoes. In the case of the studies in water, an inhibition efficiency of 100 % was observed, both with Citrocide Plus at 0.2 % and at 0.4 %, from the first 30 seconds of contact with the product, confirming this inhibition in the rest of the test with longer contact times (1, 5 and 15 minutes).

4.4.1.3 Control of microbiological contamination on the surface of the plant and vegetable products and in their treatment mixes

Three pilot tests at industrial plants were carried out in varying conditions. In all cases the doses used of the processing aid, Citrocide Plus, were 0.2 % for tomatoes and 0.4 % for citrus fruit. Total mesophilic aerobic bacteria, faecal coliform and *E. coli* counts were analysed in the treatment mixes. The surface contamination of the tomatoes and citrus fruit (lemons) by total mesophilic aerobic bacteria was also assessed.

The results obtained indicate that both in the case of tomatoes and of citrus fruit, the treatment mixes were maintained free of bacteria. In the case of surface contamination, the microbiological load is reduced by more than 2 logarithmic units of bacteria both in citrus fruit and in tomatoes.

4.4.1.4 Study of the phytotoxic effects

The applicant has submitted the results of a study, the objective of which is to establish the maximum phytotoxic dose in different varieties of citrus fruit and tomatoes.

To establish the maximum phytotoxic dose for tomatoes, increasing doses were tested on different varieties of tomatoes observing Level 2 damage at the maximum tested dose (0.23 %) in two of the varieties (Cherry and Branch tomatoes). With respect to the stain index, Level 1 stains were only observed on Cherry tomatoes. Based on the stain indexes, the application of the processing aid at doses equal to or less than 0.20 % does not represent a significant risk of phytotoxicity.

In the case of citrus fruit, Level 2 damage was only observed in the varieties of orange and mandarin when the highest studied doses were used. However, for lemons, Level 2 damage was not observed at any of the doses applied. The study concludes that considering that the minimum efficient dose of Citrocide Plus is established at 0.4 %, this dose is recommended for the washing of citric fruit in order to avoid the appearance of stains of a phytotoxic origin on the skin which would lead to a fall in the commercial quality of the treated fruit.

4.5 Description of the process

4.5.1 Ways of inserting the technological processing aid

In the application presented, the procedure for applying the processing aid Citrocide Plus is described in detail. This takes place during the washing of the fruit and vegetable products on their arrival at the packing centres, adding the processing aid to the washing water in concentrations (0.2 % for tomatoes and 0.4 % for citrus fruit) which guarantees the bacterial disinfection of the water and the citrus fruit and tomatoes.

The washing system used varies according to the product being treated. In the case of the citrus fruit, the washing mode used is the “drencher” system, or pallet shower which involves a shower system from a pallet containing 36 boxes of recently harvested fruit with a lavish flow of liquid at low pressure. The maintenance of the concentration of the mix is considered essential in this system, as it is recycled through the fruit from pallet to pallet, implying that the build-up of the contamination increases significantly with the recirculation.

With respect to tomatoes, the washing also takes place with a shower but a different device is used and it is carried out in two stages. In the first stage, tomatoes enter the prewash washer on roller beds; here they are sprayed with pressurised water which is collected in the lower section. The purpose of this stage is to remove the dirt and largest waste. Next, tomatoes go through a similar system but in addition to the shower they are also brushed. This water falls into a collection tank from where it is continuously recycled until it is no longer potable (at least once a day). The processing aid is added at this point in order to remove any existing inoculum which would infect all the tomatoes which are washed afterwards.

Automatic programmable dispensers are used for the dosage of the processing aid for citrus fruit and for tomatoes. There are also probes on the tanks which measure the concentration of peracetic acid and send the order to dispense depending on the concentration of peracetic acid, thus keeping the concentration in the washing water constant.

4.5.2 Identifying the technological processing aid elimination phases

According to the applicant, the quantities of active substances present in citrus fruits and tomatoes are insignificant given that they decompose quickly into acetic acid, water and oxygen.

With relation to peracetic acid, the 2013 report from the AECOSAN stated that the peracetic acid concentration was observed to remain or decrease slightly in the final treatment mixes used on peppers and citrus fruit due to the continuous dosage that compensated for its degradation (AECOSAN, 2013).

In the case of the stabilisers, the applicant indicated that the exposure of consumers to HEDP has not been considered, given the low concentration in the formula (<0.2 %), the low application doses (0.2 % in tomatoes and 0.4 % in citrus fruits) and their tendency to be retained in the earth and sludge which builds up during the fruit and vegetable washing process, implying that the amount of residues that may remain on the fruit as a result of this use is insignificant compared to the 4.8 mg/kg authorised by the FDA in the fruit and vegetable washing water (FDA, 2016b). In addition, in a formula with the same components but in different proportions assessed in 2013, it was found that the HEDP not only did not build up but that, unlike the DPA, it was degraded with successive treatments (AECOSAN, 2013).

On the other hand, the dipicolinic acid (DPA) builds up in the treatment mix as it is used in successive cycles.

The applicant also confirmed that both citrus fruit and tomatoes undergo a final wash with potable water before being placed on the market, thereby preventing the possible presence of residues of peracetic acid or stabilisers in these foods. In this respect, the results of pilot tests and tests carried out in the laboratory with citrus fruits and tomatoes treated with the processing aid in question are presented.

5. Analysis of residues

As indicated in the 2013 report (AECOSAN, 2013), several studies have analysed the disinfecting properties of these systems together with their toxicological properties. The JECFA have assessed the antimicrobial solutions of peroxyacids contained in HEDP (<1 %), hydrogen peroxide (4-12 %), acetic acid (40-50 %) and octanoic acid (3-10 %) in equilibrium with peracetic acid (12-15 %) and peroxyoctanoic acid (1-4 %). JECFA believes that the small quantities of residues from these peroxyacids in foods when consumed do not pose a safety threat (JECFA, 2005).

These kinds of solutions have also been assessed by the European Food Safety Authority (EFSA). EFSA (2005) assessed the use in poultry carcasses of a solution based on peroxyacids composed of peracetic acid (<15 %), peroxyoctanoic acid (<2 %), hydrogen peroxide (<10 %), acetic acid, octanoic acid and 1-hydroxyethylidene-1,1-diphosphonic acid (HEDP) (<1 %). Total peroxyacid content, expressed in peracetic acid, is 220 mg/l and the maximum concentrations of hydrogen peroxide and HEDP are 110 and 13 mg/l, respectively. In the assessment in question, aspects such as the possible toxicological risks of reaction products (for example semicarbazide) were taken into account, and it was concluded that in the conditions of use described they pose no safety threat.

As indicated in section 1, the processing aid is classified in a situation 4: substance authorised in human food for which the acceptable daily intake has not been established and the use of which may result in the technically unavoidable presence of residues in accordance with the

"Guidelines indicating the necessary documentation for the assessment of processing aids intended for use in human food" (AESAN, 2010). As a consequence, the applicant must present information on studies on residues (analytical method and validation of the method).

In relation to the possible presence of residues of the component which may build up in the treatment mixes, dipicolinic acid (DPA), in citrus fruit and tomatoes, the applicant has submitted the results of a pilot test at an industrial plant and another in a laboratory, in which the DPA concentration has been established, both in the treatment mixes and in the fruit (oranges, mandarins and tomatoes) after disinfection with the application of the processing aid.

For the determination of the DPA, a UHPLC-MS/MS method (Ultra-high performance liquid chromatography-tandem mass spectrometry) was used and validated by an external laboratory. The limits of detection and quantification are shown in table 4.

Table 4. Limits of detection and quantification of the UHPLC-MS/MS method for the determination of dipicolinic acid (DPA)

Matrices	Limit of detection (LD)	Limit of quantification (LOQ)
Oranges	0.004 mg/kg	0.1 mg/kg
Mandarins	0.004 mg/kg	0.1 mg/kg
Tomatoes	0.003 mg/kg	0.1 mg/kg
Treatment mixes	0.25 µg/l	1 µg/l

5.1 Pilot test at industrial plant

The applicant states that in order to ensure that the results were representative of the treatments applied at the fruit and vegetable packing centres, the standard washing process used for the fruit was followed, automatically applying the processing aid, to guarantee that the concentrations of the processing aid (0.4 % for citrus fruit and 0.2 % for tomatoes) were kept constant at all times.

In the case of the oranges and mandarins, an initial dose was applied at 0.4 % to start the process and subsequently the quantities of the processing aid necessary to keep the concentration levels constant were added automatically. Samples of treatment mixes were taken on each of the 10 days of the test and samples of the fruit were taken on the first day and on the last day after the final rinse with potable water.

For tomatoes, the initial dose was 0.2 % and samples were taken of treatment mixes on each of the five days of the test and samples of tomatoes (from two different varieties) were taken on the first and last day after the final rinse with potable water. The results obtained are given in Table 5.

Table 5. Contents of dipicolinic acid (DPA) in oranges, mandarins, tomatoes and treatment mixes

	DPA content					
	Oranges (mg/kg)	**Mandarins (mg/kg)**	**Treatment mixes oranges and mandarins (µg/l)**	**Pear tomatoes (mg/kg)**	**Cherry tomatoes (mg/kg)**	**Tomato treatment mix (µg/l)**
Initial mix	-	-	191.91	-	-	2.71
Day 1 start	-	-	-	n.d.	n.d.	3.3
Day 1 end	n.d.[1]	n.d.	459.31	-	-	8.56
Day 2	-	-	430.29	-	-	13.49
Day 3	-	-	655.66	-	-	11.56
Day 4	-	-	569.91	-	-	12.12
Day 5	-	-	663.79	n.d.	n.d.	34.52
Day 6	-	-	557.37			
Day 7	-	-	564.28			
Day 8	-	-	604.61			
Day 9	-	-	539.49			
Day 10	n.d.	<LOQ[2]	546.41			

[1]n.d.: not detected. [2]LOQ: limit of quantification.

5.2 Laboratory tests

The DPA concentration was determined, assuming the worst case scenario: all the DPA dispensed during a complete harvest builds up, both for citrus fruit (244.8 ppm) and for tomatoes (400 ppm). Two tests were carried out for the determination of residues in each of these plant matrices:

- Drenching oranges and tomatoes with a mix containing 100 ppm of DPA.
- Drenching oranges with a mix to which 250 ppm of DPA has been added and oranges with a mix containing 400 ppm of DPA.

In both cases the DPA content in the treatment mixes was analysed after the citrus fruit and tomato shower and the DPA content of the oranges and tomatoes was analysed before and after the final rinse with potable water. The results obtained are given in Table 6.

Table 6. Contents of dipicolinic acid (DPA) in oranges, tomatoes and treatment mixes

Matrices	DPA content in treatment mixes		DPA content in citrus fruit and tomatoes	
	DPA added (mg/l)	Content after shower (mg/l)	Before final rinse (mg/kg)	After final rinse (mg/kg)
Oranges	100	67.59	0.15	n.d.[1]
Oranges	250	204.42	0.36	n.d.
Tomatoes	100	100.03	<LOQ[2]	<LOQ
Tomatoes	400	356.19	0.488	<LOQ

[1]n.d.: not detected. [2]LOQ: limit of quantification.

5.3 Interpretation of results

Based on the data provided by the applicant, it is observed that the DPA builds up in the citrus fruit and tomato treatment mixes. In the case of the tomato treatment mixes, the DPA content increased from 2.71 μg/l (concentration in the initial mix) to 34.52 μg/l (fifth day corresponding to the last day of treatment). Whereas in the case of the mandarins and oranges, the DPA content increased from 191.91 μg/l (initial mix) to 663.79 μg/l corresponding to the fifth day of the ten days during which the treatment lasted.

However, no DPA residue was detected on oranges, mandarins and tomatoes, corresponding to the samples taken on the first and last day of the treatment. Only in the case of one sample of mandarins (taken on the last day) was the DPA residue lower than the limit of quantification (0.1 mg/kg).

In the laboratory tests, where two scenarios were considered for the citrus fruit (addition of 100 and 250 ppm of DPA), DPA residues of 0.15 and 0.36 mg/kg, respectively, were obtained before the final rinse with potable water, whereas after the final rinse the residue in both cases was insignificant.

For tomatoes, where two scenarios were also considered (addition of 100 and 400 ppm of DPA), DPA residues below the limit of quantification and 0.488 mg/kg, respectively, were obtained before the final rinse with potable water, whereas after the final rinse the residue in both cases was less than the limit of quantification.

With respect to the treatment mixes, after the treatment (shower) of the fruit, a maximum DPA concentration in orange and tomato treatment mixes of 204.42 and 356.19 mg/l, respectively were obtained.

6. Studies and data on dipicolinic acid safety

As indicated above, no ADI has been established for dipicolinic acid. An alternative involves the use of the Margin of Safety (MOS), considering that when the MOS is >100 there is no risk to the consumer. The MOS is calculated considering the NOAEL (Non Observable Adverse Effect Level) and the estimated daily intake (EDI).

In this respect, the EFSA (2009) assessed the zinc and chromium picolinates as food supplements based on the calculation of the MOS. This assessment concludes that the picolinates do not pose a risk for the consumer. Therefore, a NOAEL was established of 2 100 mg/kg b.w./day for picolinic acid based on a study conducted on rats (NTP, 2008) (EFSA, 2009) and the exposure due to the use of zinc (Zn) picolinate as a source of Zn was estimated at 1.57 mg picolinate/kg b.w./day for a person weighing 60 kg. In addition, considering the combined use of zinc and chromium picolinate as a source of Zn, the daily intake was estimated at 1.6 mg/kg b.w./day.

Another datum in favour of the innocuity of dipicolinic acid is an ingredient of the *natto,* a traditional Japanese food based on fermented soybeans (table 1). The mean daily intake of dipicolinic acid (DPA) of the Japanese population resulting from the food *natto* is 0.6-4 mg (Ohsugi and Sumi, 2011).

7. Study on consumption and evaluation of the expected level of consumer DPA intake

As established in the Guidelines for processing aids (AECOSAN, 2010), the possible presence of residues in the citrus fruit and tomatoes treated implies the need for assessment of the safety.

To estimate the exposure to DPA residue, the chronic consumption was considered in Europe of citrus fruit and tomatoes corresponding to adults and infants (0-12 months) listed in the Comprehensive European Food Consumption Database of the EFSA (2016) (tables 7 and 8). For each food, the consumption selected corresponds to the 97.5 highest percentile among those listed in the surveys included in the Comprehensive European Food Consumption Database. As additional criteria, only the data corresponding to a number of consumers >10 has been considered except in those cases for which it is the only data available.

Table 7. Adult consumption of citrus fruit and tomatoes in Europe

Food		Adult consumers	Chronic consumption (g/day)	
			Mean	**P97.5**
Tomatoes	Tomatoes	Only consumers (Romania)	97.97	310.86
	Tomato juice	Only consumers (Germany)	176.29	750
	Tomato ketchup	Only consumers (Germany)	16.66	70.60
	Tomato purée	Only consumers (Germany)	44.53	130.55
	Tomato chutney	Only consumers (United Kingdom)	10.50	57.75
Citrus fruits	Oranges	Only consumers (Finland)	163.29	540
	Orange juice	Only consumers (Germany)	216.10	850
	Mandarins	Only consumers (Italy)	94.30	350
	Lemons	Only consumers (Romania)	13.65	100
	Lemon Juice	Only consumers (Germany)	46.08	350
	Grapefruit juice	Only consumers (Germany)	233.27	790

Considering the worst possible scenario, 97.5th percentile for only adult consumers, the consumption of citrus fruit is found to be 2 980 g/day. Also considering the maximum concentration of DPA detected in the samples of citrus fruit (mandarins treated with mix from the tenth day of treatment), corresponding to the LOQ (0.1 mg/kg) of the test method used, the Estimated Daily Intake (EDI) of DPA for citrus fruit is obtained:

EDI (DPA in citrus fruit)= (2.98 kg citrus fruit/day x 0.1 mg DPA/kg)/70 kg b.w.= 0.0043 mg DPA/kg b.w./day

In the case of tomatoes, the consumption for only consumers (P97.5) in Europe is 1 319.76 g/day. Considering as the maximum concentration of DPA detected in the samples of tomatoes, the LOD of the test method used (0.003 mg/kg), an EDI is obtained for DPA in tomatoes of 0.000057 mg DPA/kg b.w./day.

On comparing the value obtained for the EDI of DPA in citrus fruit and tomatoes (0.004357 mg DPA/kg b.w./day) with the EDI obtained in the assessment of the zinc and chromium dipicolinates (1.6 mg/kg b.w./day) carried out by the EFSA (2009) and considered as safe, it is found to be far lower than this value. Just as in this assessment, the MOS concept is applied to assess the safety of the EDI of the DPA due to the consumption of citrus fruit and tomatoes treated with the processing aid:

MOS= NOAEL/EDI= 2 100/0.004357= 481 983.02>>100

The high value obtained for the MOS would indicate that there is no risk for the consumer.

An even more unfavourable scenario for the calculation of the MOS involves considering the DPA residues obtained in the laboratory tests. Considering DPA residues of 0.36 mg/kg in oranges and 0.488 mg/kg in tomatoes and a consumption (only consumers, P97.5) of 2 980 g/day for citrus fruit and 1 319.76 g/day for tomatoes, EDI of 0.015 mg DPA/kg b.w./day and 0.009 mg DPA/kg b.w./day are established respectively. Based on these EDI and a NOAEL of 2 100 mg/kg b.w./day:

MOS= NOAEL/EDI= 2 100/(0.015+0.009)= 87 500>>100

Just as in the previous case, the high value obtained for the MOS would indicate that there is no risk for the consumer.

In the case of infants aged 0-12 months, the consumption of citrus fruit and tomatoes is listed in table 8.

Table 8. Infant (0-12 months) consumption of citrus fruit and tomatoes in Europe

Food		**Infant (0-12 years) consumption**	**Chronic consumption (g/day)**	
			Mean	**P97.5**
Tomato	Tomato	Only consumers (Finland)	12.51	75
	Tomato juice	Only consumers (Bulgaria)	80	80
	Tomato ketchup	Only consumers (United Kingdom)	2.25	7.5
	Tomato purée	Only consumers (United Kingdom)	4.62	24.75
Citrus fruits	Oranges	Only consumers (Denmark)	12.63	51
	Orange juice	Only consumers (Bulgaria)	61.13	175
	Mandarins	Only consumers (United Kingdom)	13.71	68.75
	Lemons	Only consumers (Bulgaria)	2.10	5.34
	Lemon Juice	Only consumers (United Kingdom)	0.65	2.40
	Grapefruit juice	Only consumers (Bulgaria)	15	15

Considering the worst case scenario, P97.5 for only infant consumers (0-12 months), the consumption of citrus fruit and tomatoes is found to be 317.49 g/day and 187.25 g/day, respectively.

As in the case of the adults, considering the maximum concentration of DPA detected in the samples of citrus fruit (mandarins treated with mix from the tenth day of treatment), corresponding to the LOQ (0.1 mg/kg) of the test method used, the Estimated Daily Intake (EDI) of DPA for citrus fruit is obtained:

EDI (DPA in citrus fruit)= (0.32 kg citrus fruit/day x 0.1 mg DPA/kg)/5 kg b.w.= 0.0064 mg DPA/kg b.w./day

In the case of tomatoes, considering as the maximum concentration of DPA detected in the samples of tomatoes, the LOD of the test method used (0.003 mg/kg), an EDI is obtained for DPA in tomatoes of 0.000114 mg DPA/kg b.w./day.

On comparing the value obtained for the EDI of DPA in citrus fruit and tomatoes (0.006514 mg DPA/kg b.w./day) with the EDI obtained in the assessment of the zinc and chromium dipicolinates (1.6 mg/kg b.w./day) carried out by the EFSA (2009) and considered as safe, it is found to be far lower than this value. As for the case of the adults, the MOS concept is applied to assess the safety of the EDI of the DPA due to the consumption of citrus fruit and tomatoes treated with the processing aid, obtaining MOS= 322 382.6>100

The high value obtained for the MOS would indicate that there is no risk for the consumer.

As for the adults, an even more unfavourable scenario for the calculation of the MOS involves considering the DPA residues obtained in the laboratory tests. Considering DPA residues of 0.36 mg/kg in oranges and 0.488 mg/kg in tomatoes and a consumption (only consumers, P97.5) of 317.49 g/day for citrus fruit and 187.25 g/day for tomatoes, EDI of 0.023 mg DPA/kg b.w./day and 0.019 mg DPA/kg b.w./day are established respectively. Based on these EDI and a NOAEL of 2 100 mg/kg b.w./day:

MOS= NOAEL/EDI= 2 100/(0.023+0.019)= 50 000>100

Similarly, it should be noted that as established in the conditions of use, both the citrus fruit and tomatoes receive a final rinse with potable water, after which the DPA residues are not detectable (<0.004 mg/kg) in oranges and below the limit of quantification (0.1 mg/kg) in the case of tomatoes, as indicated in the tests submitted by the applicant.

The same conclusion regarding the absence of risk for the consumer can be reached by comparing the EDI for the DPA in citrus fruit and tomatoes in adults and children (0.004357 mg DPA/kg b.w./day and 0.006514 mg DPA/kg b.w./day, respectively) with the mean daily intake of DPA of the Japanese population resulting from the intake of the food *natto* which is 0.01-0.066 mg/kg b.w./day.

Conclusions of the Scientific Committee

The Scientific Committee, having assessed the application file for use of this technological processing aid in the process of bacterial disinfection of citrus fruits and tomatoes as well as washing waters, concludes that, based on the information provided by the applicant and the proposed dosage and conditions, there is no risk posed to consumer safety.

The conclusions of this report refer exclusively to the product under assessment as a processing aid under the conditions of use proposed and with its current composition, both as regards its active components and its stabilisers. They cannot be extrapolated to other formulae or conditions other than those assessed. It should be remembered that the kg of fruit treated, the climatic conditions and the dirt may affect the concentrations of the components of the processing aid in the treatment mixes and therefore in the possible residues.

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