United States Department of Agriculture
Agricultural Marketing Service | National Organic Program
Document Cover Sheet
https://www.ams.usda.gov/rules-regulations/organic/national-list/petitioned

Document Type:

☐ **National List Petition or Petition Update**

A petition is a request to amend the USDA National Organic Program's National List of Allowed and Prohibited Substances (National List).

Any person may submit a petition to have a substance evaluated by the National Organic Standards Board (7 CFR 205.607(a)).

Guidelines for submitting a petition are available in the NOP Handbook as NOP 3011, National List Petition Guidelines.

Petitions are posted for the public on the NOP website for Petitioned Substances.

☒ **Technical Report**

A technical report is developed in response to a petition to amend the National List. Reports are also developed to assist in the review of substances that are already on the National List.

Technical reports are completed by third-party contractors and are available to the public on the NOP website for Petitioned Substances.

Contractor names and dates completed are available in the report.

Sodium Citrate

Crops

Identification of Petitioned Substance

Chemical Names:

Monosodium citrate, disodium citrate, trisodium citrate, sodium citrate

Other Name:

Sodium dihydrogen citrate, disodium hydrogen citrate, Trisodium 2-hydroxypropane-1,2,3-tricarboxylate

Trade Names:

Citrosodina, Natrocitral, Citnatin, Orange Eno

CAS Numbers:

18996-35-5;
144-33-2;
68-04-2;
6132-04-3; 6858-44-2

Other Codes:

Pubchem ID: 6224; InChI Key: HRXKRNGNAMMEHJ-UHFFFAOYSA-K
InChI: InChI=1S/C6H8O7.3Na/c7-3(8)1-6(13,5(11)12)2-4(9)10;;;/h13H,1-2H2,(H,7,8)(H,9,10)(H,11,12);;;/q;3*+1/p-3
Canonical SMILES: C(C(=O)[O-])C(CC(=O)[O-])(C(=O)[O-])O.[Na+].[Na+].[Na+]
EC Number: 200-675-3, 218-618-2
FEMA Number: 3026
ICSC Number: 1218
RTECS Number: GE8300000
UNII: RS7A450LGA

Summary of Petitioned Use

Sodium citrate is used as an anticoagulant in the collection of slaughterhouse blood. Slaughterhouse blood is used to make the soil amendment, blood meal. Slaughterhouse blood can be processed in different ways to make blood meal. The petition requests the addition of sodium citrate to the National List (§ 205.601), allowed for use in crop production to prevent animal blood coagulation after collection and during processing of blood for production of blood meal.

Characterization of Petitioned Substance

Composition of the Substance:

Sodium citrate is a soluble white powder. It has many uses. One of which is as an anticoagulant in the collection and processing of animal blood. Sodium citrate treated blood may be used for production of the soil amendment, blood meal. Animal blood meal is allowed in organic crop production as a soil amendment.

Source or Origin of the Substance:

Sodium citrate is a salt derivative of citric acid. Citric acid is naturally occurring. Sodium citrate is chemically produced by the same process as citric acid (NOP, 2015). Commercially, citric acid is produced microbiologically mostly from the sugar refinery byproduct, molasses. The mycelial fungus *Aspergillus niger* or *Candida* spp. yeasts are frequently used for these fermentation processes. Citric acid from fermentation is neutralized with sodium hydroxide and crystalized in the production of sodium citrate. Sodium citrate can be produced microbiologically, directly from cultures of the yeast *Yarrowia lipolytica*, since this organism can tolerate a higher pH (Kamzolova et al., 2015).

Sodium citrate is routinely added to blood as it is removed from animal carcasses during processing. The addition of sodium citrate keeps blood flowing and minimizes extensive cleaning of clotted blood from extraction and collection equipment. Anticoagulants have been considered incidental to blood meal production and part of the standard identity for blood, since a substantial portion of added sodium citrate is removed during manufacturing. Furthermore, it may not be reliably possible for manufacturers to

determine if anticoagulants have been added to blood for blood meal production (Bungum, 2017). Animals can be bled, and their blood collected without the addition of sodium citrate. This practice is not common for large animal processing plants (Food Safety Authority of Ireland, 2013; NCPS Board of Consultants and Engineers, 2016).

Properties of the Substance:

Sodium citrate, the sodium salt derivative of citric acid, is a crystalline white powder with a melting point of >300°C. Its molecular formulae are: anhydrous: $C_6H_5O_7Na_3$; hydrated: $C_6H_5O_7Na_3 \cdot nH_2O$ (n = 2 or 5) or $C_6H_5Na_3O_7$ or $C_6H_5O_7$. 3Na. It has a molecular weight of 258.08 grams/mole. A two-dimensional structure of sodium citrate is provided in Figure 1. Previous technical reviews for citric acid and sodium citrate are available on the NOP website (NOP, 2015).

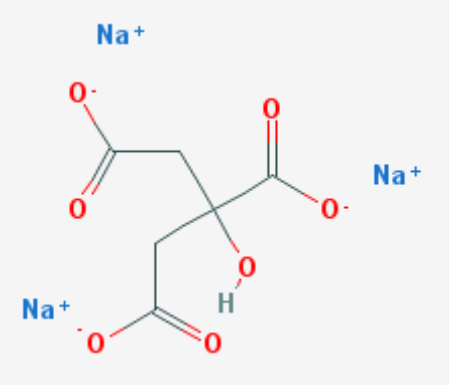

Figure 1 2D Sodium Citrate Structure

(PubChem, 2017)

Specific Uses of the Substance:

Sodium citrate is routinely used as an anticoagulant for blood collection during slaughtering and processing of livestock. It may be applied to the sticking knife, to improve blood flow during bleeding or added to collection or storage tanks to improve stability. Blood products are separated, cooked and dried into powder at the meat processing plant or further processing plants. Storage and transfer of blood requires refrigeration. Blood meal, a by-product of the animal slaughtering industry is used in organic crop production as a soil amendment (Yunta et al., 2013; §205.203(c)). Several methods are in use commercially for production of blood meal. These differ in clotting or no clotting, drying steps and the separation of red blood cells. Some examples are batch dried, ring dried and spray dried rendering. Batch dry rendering is simple cooking of whole blood with indirect high-pressure steam to remove moisture. Ring dried rendering requires coagulation and separation of the coagulated blood from fluids. The coagulum is separately dried. In spray drying, which requires the use of sodium citrate, flowing blood treated with anticoagulant is sprayed into a warm chamber where it instantly becomes a fine powder. Drying method affects the characteristics and quality of the final product. With meat inspection, blood meal can also be used for conventional human and animal nutrition. In addition to simply drying clotted whole blood, blood may be fractionated during processing to separate red blood cells from plasma or remove specific higher valued products before dried meal is produced.

Approved Legal Uses of the Substance:

Sodium citrate has been verified to be of low concern based on experimental and modeled data for use as a chelating agent (anticoagulant), a preservative, an antioxidant, a processing aid and an additive (EPA Safer chemical ingredients list). Sodium citrate is included in the FDA list of substances generally regarded as safe. It is the sodium salt of citric acid prepared by fermentation and neutralization of citric acid with sodium hydroxide or sodium carbonate. The product occurs as colorless crystals or a white crystalline powder. It may be prepared in an anhydrous state or may contain two moles of water per mole of sodium citrate (21 CFR 184.1751). Sodium citrate is listed in the National List as an allowed synthetic for use in organic handling (§205.605b). The sodium salts of citric acid – monosodium citrate, disodium citrate and tri sodium citrate – are collectively listed as "sodium citrate." These substances are used similarly as pH

control/buffering agents and stabilizers in food products. The original technical review found sodium citrate to be consistent with the OFPA 2119(m) criteria (NOSB, 2010). Sodium citrate is not allowed for use in organic crop production.

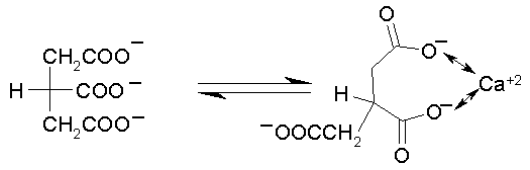

Figure 2 Chelation of Ca^{++} by Sodium Citrate

Action of the Substance:

Blood is an important meat animal processing byproduct. Blood meal, a non-synthetic product of animal byproduct processing, is allowed for use as a soil amendment in organic crop production (205.203(c)). Approximately 4-5% of live animal weight is collectable blood which contains approximately 10% of animal protein. When fresh blood is extracted from an animal, fibrinogen in the blood is converted to fibrin. The presence of fibrin catalyzes the formation of a fibrous network that enmeshes blood cells and other blood components into a clot. Clotting can be inhibited by vigorous agitation, chilling or by the addition of anticoagulants. Sodium citrate is an anticoagulant commonly used for collecting blood in slaughterhouses (Fernando, 1992). Ionic calcium is essential for the conversion of fibrinogen to fibrin. Sodium citrate acts to chelate or remove available calcium required for the fibrinogen to fibrin conversion preventing blood coagulation (clotting). In chelation, calcium binds to the dentate carboxyl moieties of citrate (Fig. 2).

Blood can become recalcified through cell breakdown and bacterial degradation. When calcium is available for fibrinogen to fibrin conversion, clotting resumes. After bleeding warm blood is only stable for approximately eight hours. Without refrigeration, fresh whole blood must be processed and dried shortly after bleeding. Even with the addition of sodium citrate, animal byproduct producers reduce whole blood degradation, bacterial contamination and further clotting by chilling stored blood with stirring prior to inspection and further downstream processing. This is important, if blood must be transported to another facility. Chilled whole blood held at 2-3°C is stable for approximately 120 hours which facilitates off site processing (Labudde Group, 2017; Sjoberg, 2017).

Combinations of the Substance:

Sodium citrate is added directly to blood as it is collected during meat animal processing. It may be dissolved in water and added as a solution to speed its action. Other substances are not generally used in combination for byproduct meat animal blood processing.

Status

Historic Use:

Sodium citrate was first used as an experimental anticoagulant in blood transfusion for dogs in the 1890s (Mollison, 2000; Hedley-Whyte and Miamed, 2010). By 1915, the minimum amount of sodium citrate necessary for anticoagulation of blood without side effects had been determined for human use (Lewisohn, 1915). By 1918, the military development of an acceptable procedure for human blood transfusion and blood storage became a necessity. Sodium citrate at 0.2% was not only safe for humans use, but could be used for routine transfusion practice and storage of whole blood for up to two weeks (Arthus, 1905; Lewisohn, 1918). Sodium citrate has been used as an anticoagulant for the collection of slaughterhouse blood since the late 1800s (Wismer-Pedersen, 1988).

Organic Foods Production Act, USDA Final Rule:

Sodium citrate is listed on 205.605(b), synthetics allowed for processed products labeled as organic.

International

Canada - Canadian General Standards Board Permitted Substances List. Sodium citrate is listed in CAN/CGSB-32.311-2015 — Organic production systems - Permitted substances lists sodium citrate as a food additive, as a food grade cleaner, disinfectant and sanitizer (without removal), and as a cleaner, disinfectant and sanitizer (removal is mandatory).

CODEX Alimentarius Commission, Guidelines for the Production, Processing, Labelling and Marketing of Organically Produced Foods (GL 32-1999) -

According to Codex Alimentarius GL 32-1999, sodium citrate is not permitted for use in organic production of food of plant origin, but is permitted for use in organic production in processed food of animal origin as follows: butter milk (plain) (stabilizer only); dairy-based drinks, flavored and/or fermented (e.g., chocolate milk, cocoa, eggnog, drinking yoghurt, whey-based drinks); fermented milks (plain), heat-treated after fermentation (stabilizer only); renneted milk (stabilizer only); condensed milk and analogues (plain) (stabilizer only); cream (plain) and the like (stabilizer only); milk powder and cream powder (plain) (stabilizer only); unripened cheese (stabilizer only); processed cheese (emulsifier only); dried whey and whey products, excluding whey cheeses; processed comminuted meat, poultry, and game products, restricted to sausages; to be used in pasteurization of egg whites only in the following: egg products.

European Economic Community (EEC) Council Regulation, EC No. 834/2007 and 889/2008

Commission Regulation (EC) No 889/2008 lays down rules for the use of sodium citrate. It is permitted in the production of processed organic food for preparation of foodstuffs of animal origin, but not permitted in foodstuffs of plant origin.

Japan Agricultural Standard (JAS) for Organic Production—

The Japanese Agricultural Standard for Organic Processed Foods allows the use of sodium citrate limited to dairy products or albumen and sausage as low temperature pasteurization. The Japanese Agricultural Standard for organic livestock does not allow the use of sodium citrate. The Japanese Agricultural Standard for organic plants does not allow the use of sodium citrate. The Japanese Agricultural Standard for organic feeds does not allow the use of sodium citrate.

International Federation of Organic Agriculture Movements (IFOAM) –

The IFOAM norms allow the use of sodium citrates for production of processed foods as an additive and as a processing aid.

Evaluation Questions for Substances to be used in Organic Crop or Livestock Production

Evaluation Question #1: Indicate which category in OFPA that the substance falls under: (A) Does the substance contain an active ingredient in any of the following categories: copper and sulfur compounds, toxins derived from bacteria; pheromones, soaps, horticultural oils, fish emulsions, treated seed, vitamins and minerals; livestock parasiticides and medicines and production aids including netting, tree wraps and seals, insect traps, sticky barriers, row covers, and equipment cleansers? (B) Is the substance a synthetic inert ingredient that is not classified by the EPA as inerts of toxicological concern (i.e., EPA List 4 inerts) (7 U.S.C. § 6517(c)(1)(B)(ii))? Is the synthetic substance an inert ingredient which is not on EPA List 4, but is exempt from a requirement of a tolerance, per 40 CFR part 180?

As an anticoagulant used in processing blood for blood meal, sodium citrate may be considered a production aid (7 USC 6517(c)(1)(B)(i)). Sodium citrate is the sodium salt of citric acid prepared from citric acid by neutralizing citric acid with sodium hydroxide or sodium carbonate followed by a crystallization step. Commonly available forms are anhydrous or dehydrate.

Evaluation Question #2: Describe the most prevalent processes used to manufacture or formulate the petitioned substance. Further, describe any chemical change that may occur during manufacture or formulation of the petitioned substance when this substance is extracted from naturally occurring plant, animal, or mineral sources (7 U.S.C. § 6502 (21)).

Sodium citrate is produced by addition of sodium carbonate monohydrate to a hot aqueous solution of citric acid. The resulting solution is then evaporated until crystallization has taken place. Another synthetic method used for producing sodium citrate is decomposing calcium citrate with an alkali metal salt (sodium). Citric acid production is described in a 2015 NOP technical report. Some microorganisms can produce sodium citrate directly during fermentation. Sodium citrate is directly recovered from citric acid fermentation broth by removing impurities at pH 9-13 and concentrating the resulting fluid at pH 10-13. The organisms for this type of fermentation are yeasts, such as *Candida, Bretanomyces, Debaryomyces, Hanseula, Koeckera, Torulopsis, Pichia, Triospora, Saccharomyces* and bacteria such as *Corynebacterium* and *Arthrobacter* (Tsuda et al., 1975). In another process, *Yarrowia lipolytica* ferments glycerol-containing biodiesel waste and produces sodium citrate, which is filtered from the culture after pH adjustment to 7-8 with NaOH (Kamzolova et al., 2015).

Evaluation Question #3: Discuss whether the petitioned substance is formulated or manufactured by a chemical process, or created by naturally occurring biological processes (7 U.S.C. § 6502 (21)).

Sodium citrate is synthetic. It is currently classified as synthetic in 205.605(b). The use of sodium citrate as an anticoagulant depends on the application and process approach. When a farm animal is slaughtered blood is released in an amount equivalent to 6-7% of the lean meat of the carcass based on total protein. Many cultures consider meat animal blood a food (Wismer-Pedersen, 1988). In addition to uses in food, animal blood has many uses in feed, laboratory, medical, industrial and fertilizer applications (Ockerman and Hansen, 2000).

Blood is composed of two primary fractions separable by centrifugation: the plasma and the red blood cells. Red blood cells contain the protein hemoglobin (Fernando, 1992). A relatively small quantity of white blood cells and platelets are also present. Plasma contains the proteins albumin, globulin and fibrinogen. Fibrinogen is involved in clotting. Greater than 80% of raw blood is water (Fernando, 1992).

The efficiency of blood collection depends on the animal, the length of time permitted for bleeding and the method for collection (Fernando, 1992). Blood from slaughterhouse animals is usually collected in one of two ways depending upon the application. It can be collected hygienically for use in foods and products, such as hemoglobin and plasma proteins. A closed draining system can be used where blood from the slaughterhouse animal is not exposed to air and is drained directly from the body of the animal; for example, using a hollow knife connected to vacuum piping (Fig 3). Blood for food or therapeutic applications must come with a guarantee that it is sourced from veterinary-approved disease-free animals and is free from contamination. In alive and healthy animals, blood is “sterile”, in the sense that it can be consumed. However, collecting blood hygienically requires additional equipment, adds cost and slows down any slaughtering line speed (Bah et al., 2013). Transport of harvested blood to a processing facility may also require the use of a refrigerated tanker truck (Fernando, 1992). Another method for collecting animal blood is open draining into buckets, trays or onto the floor. This method is particularly susceptible to contamination and not likely to be suitable for food or therapeutic applications. Rather blood collected this way is used industrially or for fertilizer production. In any case it is prudent to consider collecting blood as a byproduct rather than discarding it. Blood has a high chemical oxygen demand (COD) (500,000 milligrams O_2/liter). As a result, disposal of large quantities of slaughterhouse blood can cause environmental problems (Kostic et al., 2013).

After bleeding clotting takes place in three to ten minutes depending on the environmental temperature. Clotting is caused by the conversion of soluble fibrinogen in the blood to insoluble fibrin by the enzyme thrombin. Clotting does not occur in circulating blood because there are natural anticoagulants present in intact blood vessels. Clotting may or may not be desirable for processing depending on the use of collected blood (Fig 4). Some of the commercial processes used for production of blood meal, which is used as a soil amendment in organic crop production require blood to clot to separate the solids from water. However, blood is a complex product and some value-added production streams may require the use anticoagulants to permit collection and separation of erythrocytes and protein products in addition to the production of blood meal. Clotting can be efficiently inhibited with the addition of 0.2 % sodium citrate during blood collection (Lewisohn, 1915). Regulations for the use of sodium citrate in the food and pharmaceutical industry vary from country to country (Ockerman and Hansen, 2000). Sodium citrate removes ionic calcium from solution. Ionic calcium is necessary for clotting to occur (Kingston et al., 2001).

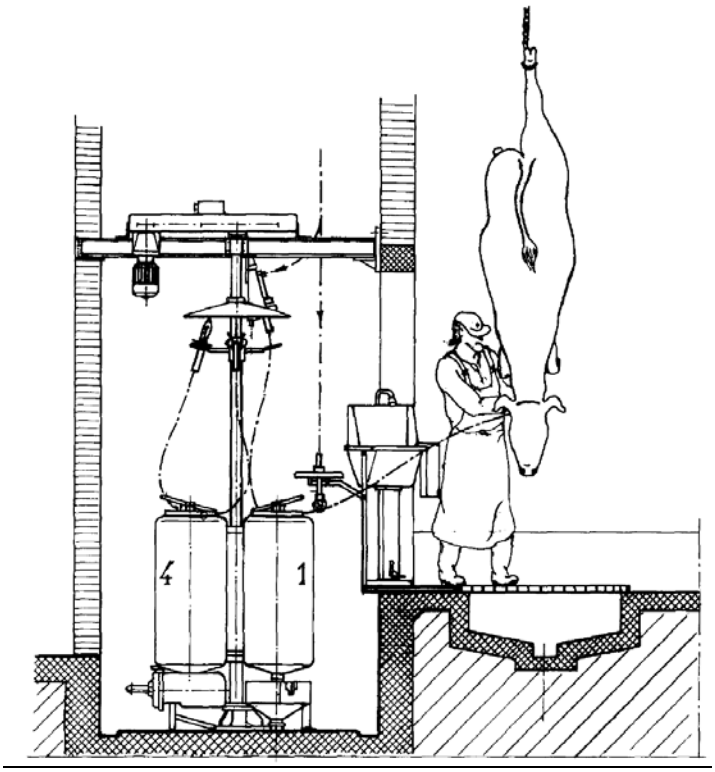

Fig. 3 Example of equipment to hygienically collect blood in an abattoir (*from* Wismer-Pedersen, 1988).

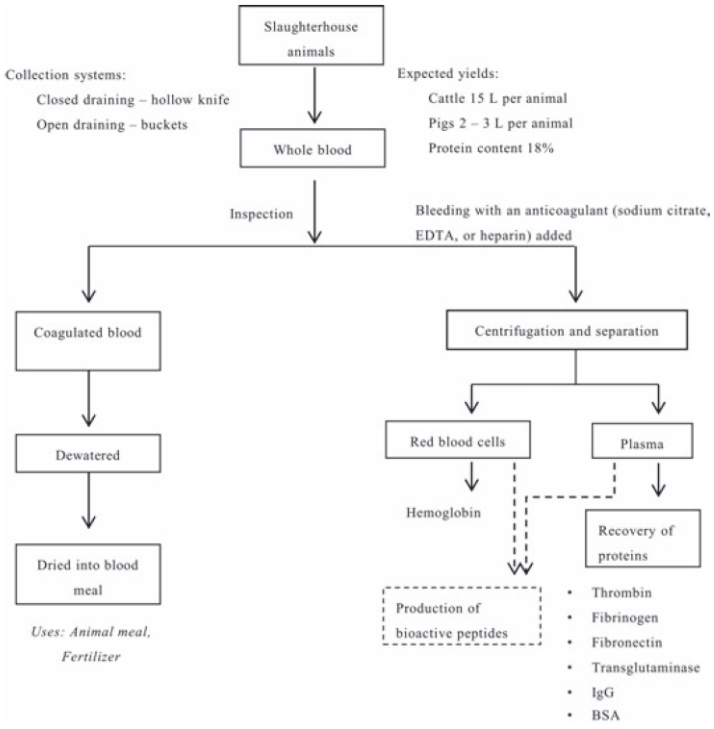

Fig. 4 Treatment of slaughterhouse blood for specific uses (*from* Fernando, 1992)

Sodium citrate is an allowed synthetic substance for use as an ingredient in organic processing (205.605(b)). Sodium citrate is not on the National List for use in organic crop production.

Evaluation Question #4: Describe the persistence or concentration of the petitioned substance and/or its by-products in the environment (7 U.S.C. § 6518 (m) (2)).

Sodium citrate is the sodium salt of citric acid. It is highly mobile in the environment and partitions to the aquatic compartment. Sodium citrate is rapidly degraded microbiologically in sewage works, in surface waters and in soil. Generally, citric acid and its salts have not been judged by the EPA or Organization for Economic Cooperation to be substances that present a hazard to the environment (EPA, 1992; OECD, 2001).

Evaluation Question #5: Describe the toxicity and mode of action of the substance and of its breakdown products and any contaminants. Describe the persistence and areas of concentration in the environment of the substance and its breakdown products (7 U.S.C. § 6518 (m) (2)).

Sodium citrate is of low acute toxicity to freshwater fish, daphnia, algae and marine species. Similarly, sodium citrate has no obvious toxic potential against protozoans and many species or strains of bacteria including activated sludge micro-organisms (EPA, 1992; OECD, 2001).

Evaluation Question #6: Describe any environmental contamination that could result from toxicity due to the petitioned substance's manufacture, use, misuse, or disposal (7 U.S.C. § 6518 (m) (3)).

Sodium citrate is produced biologically by the same submerged fermentation process with starch/sucrose-based media as citric acid, but is neutralized in the presence of appropriate alkaline solutions (e.g., sodium hydroxide or sodium carbonate) and crystallized. Several agricultural waste residues and by-products are used as production substrates for sodium citrate production including molasses, fruit pomace waste, wheat bran, coffee husk, and cassava bagasse. Most of the substrates would otherwise be composted, but represent a value-added component in sodium citrate production (Dhillon et al., 2011). Fermentation waste can be composted. However, the production of 1 ton of citric acid produces 40 tons of acidic wastewater with a high chemical oxygen demand. Production wastewater can be treated by biohydrogen production, electrochemical oxidation, membrane filtration and anaerobic and aerobic bacterial digestion. Studies are underway to repurpose this wastewater stream for methane production (Zhang et al., 2014).

Evaluation Question #7: Describe any known chemical interactions between the petitioned substance and other substances used in organic crop or livestock production or handling. Describe any environmental or human health effects from these chemical interactions (7 U.S.C. § 6518 (m) (1)).

Sodium citrate is very soluble in water and microbiologically degradable. As an anticoagulant for slaughterhouse blood, sodium citrate is used at a concentration of 0.2-0.4% and may become a component of the meat processing effluent. As a low concentration component (≤0.08%) of blood meal used as a soil amendment it is expected for sodium citrate to become a metabolite of soil bacteria.

Evaluation Question #8: Describe any effects of the petitioned substance on biological or chemical interactions in the agro-ecosystem, including physiological effects on soil organisms (including the salt index and solubility of the soil), crops, and livestock (7 U.S.C. § 6518 (m) (5)).

Sodium nitrate is used at a concentration of 0.2-0.4% in whole fresh blood. Blood is mostly composed of water (≥80%). Thus, dried blood meal is expected to contain no more than ~0.1% sodium citrate. Potential organic fertilizer nitrogen sources vary in nitrogen cost and nitrogen mineralization rate. Blood meal has a nitrogen content of about 12% and 75% of organic carbon and nitrogen is mineralized after 8 weeks at 25°C. The rest can be found in humus components (Ciavatta et al., 1997). Blood meal is comparable to liquid fertilizers, e.g. liquid fish (Gaskell and Smith, 2007). It can be prepared by spray drying hemolyzed red blood cells from sodium citrate treated slaughterhouse blood and is a good soil amendment for the prevention of iron chlorosis in plants (Gruppo Farpro, 2017; Kalbasi and Shariatmadari, 1993). Mossbauer and electron paramagnetic spectra revealed that iron from the blood meal amendment is associated with the porphyrin heme group of hemoglobin. There is an advantage to application of iron in blood meal since it is bound to an organic moiety easing plant uptake of iron. However, when high $CaCO_3$ is present in the soil, the iron bound porphyrin is likely to aggregate and cause the iron to be retained in the soil. Sodium citrate does not appear to negatively affect soil fertility (Yunta et al., 2013). As a fertilizer, blood meal produced using sodium citrate treated blood, provides sources of nitrogen, phosphorus, and calcium;

improves soil structure; promotes beneficial soil microorganisms; encourages earthworms; increases plant growth and yield; provides a balanced supply of nitrogen, phosphorus, and potassium, and organic matter including amino acids, albumin, globulin, cholesterol, and calcium; increases the growth promoters tricontanol and gibberellic acid; reduces waterlogging plant stress and reduces plant stress recovery time (Quilty and Cattle, 2011). Application of blood meal as soil amendment causes soil electrical conductivity, organic matter and pH to increase (Citak and Sonmez, 2011).

Evaluation Question #9: Discuss and summarize findings on whether the use of the petitioned substance may be harmful to the environment (7 U.S.C. § 6517 (c) (1) (A) (i) and 7 U.S.C. § 6517 (c) (2) (A) (i)).

Sodium citrate is the sodium salt of citric acid. Citric acid has been produced for many years in high volumes and added to processed food and beverages, used in pharmaceutical preparations and in household cleaners as well as in special technical applications (OECD, 2000). Citric acid is a well-known component of carbohydrate metabolism in living organisms, and is found naturally in soil and water. It degrades readily when in contact with a variety of microorganisms that are found in soil, natural waters and sewage treatment systems (EPA, 1992). Citric acid is of low acute toxicity to freshwater fish, daphnia and algae and a few marine species, e.g. crabs, green algae, diatoms. Similarly, citric acid has no obvious toxic potential against protozoans and many species or strains of bacteria including activated sludge micro-organisms. Monitoring data has shown that while raw sewage contains up to 10 milligrams citrate/liter, background concentrations in river water range between < 0.04 and maximally 0.2 mg/l, and between 0.025 and 0.145 mg/l in Atlantic coast surface seawater. However, these water concentrations for citrate do not only arise from manmade citric acid. Citric acid is extremely widespread in plant and animal tissues and fluids and every single eukaryotic organism produces citric acid and excretes part of it to the environment. Based on a large volume of available data collected by the Organization for Economic Development citric acid was not judged to be a substance that presents a hazard to the environment (OECD, 2000).

Evaluation Question #10: Describe and summarize any reported effects upon human health from use of the petitioned substance (7 U.S.C. § 6517 (c) (1) (A) (i), 7 U.S.C. § 6517 (c) (2) (A) (i)) and 7 U.S.C. § 6518 (m) (4)).

Industrial releases of citric acid can occur from the production site and its use in industrial processes. Consumers are directly exposed to citric acid or its salts in diluted concentrations in many products from soft drinks and processed food to common household cleaners, detergents and washing powders. There is no acceptable daily intake level. Occupational exposure may occur during manufacturing and processing of sodium citrate. There is no recommended occupational exposure level. Citric acid has a low acute toxicity by oral application in both rat (LD50 = 3,000– 12,000 mg/kg, 3 different values) and mouse (LD50 = 5,400 mg/kg). General effects consisted of physiological disturbances (acidosis and calcium deficiency), while "high" doses caused nervous system effects as well as severe damage to the stomach mucosa. By subcutaneous application, LD50 values of 5,500 mg/kg in rats and 2,700 mg/kg in mice have been reported. Injection of citric acid by various routes in rats, mice and rabbits (no doses stated) caused nervous system, lung, spleen and liver effects that were in part attributed to acidosis and calcium deficiency. Ingestion of a single dose of 25 g of citric acid by a woman (corresponding to approx. 417 mg/kg) caused vomiting and near dying in one reported case. Volunteers given oral doses of potassium or magnesium citrate corresponding to approx. 4.7 g of citric acid did not suffer any overt gastrointestinal effects. Injection of large volumes of citrated blood during transfusion may lead to hypocalcaemia and changes in blood composition with concomitant nausea, muscle weakness, breathing difficulties and even cardiac arrest. Sodium citrate is a strong irritant to the eyes and a moderate skin irritant (OECD, 2000).

Evaluation Question #11: Describe all natural (non-synthetic) substances or products which may be used in place of the petitioned substance (7 U.S.C. § 6517 (c) (1) (A) (ii)). Provide a list of allowed substances that may be used in place of the petitioned substance (7 U.S.C. § 6518 (m) (6)).

There are not many non-synthetic substances or products that may be used as anticoagulants for slaughterhouse blood processing. Such substances would need to prevent the proenzyme, thrombinogen from converting to thrombin, prevent the proenzyme fibrinogen from converting to fibrin and/or prevent the web-like matrix formation of fibrin in the blood, e.g. chelating calcium ions. Naturally, the glycoprotein heparin serves as an anticoagulant in blood vessels and in the intestines. Because heparin is chemically

extracted from animal byproducts and crystallized as a salt, it is not considered non-synthetic. Heparin is prohibited for use in livestock care (205.105(a)). A mixture of phosphates containing 22% Na_2HPO_4, 22%$Na_4P_2O_7$, 16% $Na_2H_2P_2O_7$ and 40% NaCl at a rate of 10 grams/liter is an effective anticoagulant, although they are not included in section 205.601 of the National List and not allowed for use in organic crop production. Sodium oxalate may also be used as an anticoagulant, but it is considered poisonous and may not be appropriate for application to soil as a soil amendment (Ockerman and Hansen, 2000).

Plant, bacterial and fungal proteolytic enzymes such as papain, bromelin, trypsin, fibrinolysin, bacterial protease N, bacterial protease P, bacterial protease S and others have been used in place of anticoagulants industrially to extract proteins from blood. These enzymes act proteolytically on fibrin to prevent clotting and support a process to provide good quality protein (Quaglia and Massacci, 1982).

Evaluation Question #12: Describe any alternative practices that would make the use of the petitioned substance unnecessary (7 U.S.C. § 6518 (m) (6)).

In practice, blood flows from an animal after it is stuck with a knife (Fig 3). The blood can be collected in troughs or tanks beneath the animal. If a hollow knife is used with an anticoagulant injected at knifepoint whole blood can be pumped aseptically to tanks for further processing. Further processing can include conventional use in foods and feed if the animal carcass from which it came is approved by a meat inspector. An anticoagulant can also be added to the open troughs or tanks to facilitate additional separations, e.g. whole blood may be separated into red blood cells and plasma and the fractions are dried or processed separately. Separated red blood cells can be dried or spray dried for use in blood meal for fertilizer.

Without added anticoagulant, clotted blood is collected and processed by separating clotted blood from the water component, drying and grinding. (Stevenson and Lloyd, 1979). Blood that is collected in this way can be directly batch dried. In this drying process, water may be added to the blood as it is charged into a batch cooker that simply dries the blood to 2-10% moisture. In batch coagulation followed by batch drying raw blood is first coagulated with steam. The coagulum is then separated by draining off liquid before it is moved to a drier for drying. Continuous coagulation before drying is the most commonly used process. In each of these processes, an anticoagulant is optional (Fernando, 1992). Rapid chilling of blood to 1-2^{o} C (34-36^{o}F) will prevent coagulation without an anticoagulant, but blood will coagulate when the temperature increases. Agitation and refrigeration are routinely used where blood must be stored or transported prior to processing to prevent microbial growth. Vigorous stirring of blood causes fibrin to adhere to the stirring rod and prevent coagulation, however this process damages red blood cells (Ockerman and Hansen, 2000). This process called defibrination removes the potential of blood to clot. Defibrinated blood is available commercially.

Blood is an edible byproduct of meat processing. Edible blood is regulated in the same way as other meat products and must be inspected prior to consumption by the supervising agency. Edible by-products are perishable and must be chilled quickly after slaughter and processed or moved into retail trade (Ricke et al., 2012). One certified organic slaughterhouse in the US provides blood for human consumption (Kaufman, 2015; Organic Integrity Database (Operation Profile (7360000108) updated on 12/14/2017)). Sodium citrate may be added to fresh whole blood collected for human consumption. Dried blood as a food grade ingredient may contain less than 0.1% of sodium citrate by weight. Producers must usually follow hazard analysis critical control point (HAACP) principles, clean equipment after each use and document the origin of each batch of blood. Regardless of whether or not an anticoagulant is used, storage of fresh blood is maintained with stirring and chilling in closed containers (Food Safety Authority of Ireland, 2013). Chilling in this case in this case also inhibits the growth of bacterial contaminants.

Labels for blood meal advertised for use as fertilizer do not normally indicate the animal origin of the product, the condition of the animals, whether an anticoagulant (e.g. sodium citrate) was used or the process that was used for production. Thus, unless specifically stated on the label, it may not be possible to determine if sodium citrate was used as an anticoagulant during the collection of blood to be used for blood meal. There are no organic production operations listed in the organic integrity database for 2017 that are certified to provide organically produced blood for food or fertilizer.

Slaughterhouse blood processing end products' technical and sanitary requirements determine their costs and production efficiencies. Lots that are rejected for a higher priced product may be acceptable for another

less expensive product. Specifically, reliable sourcing of blood meal prepared from slaughterhouse blood that was not treated with sodium citrate may require traceabilty and segregation of the non-treated material after it was withdrawn from animals independently of how the blood meal was prepared. Such information could be provided on the product label or obtained from a process verification audit.

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