Gibberellic Acid

Handling/Processing

Identification of Petitioned Substance

Chemical Names:
(3S,3aR,4aS,7S,9aR,12S)-7,12-dihydroxy-3-methyl-6-metylene-2-oxoperhydro-4a,7-methano-9b,3-propenoazuleno[1,2-b]-furan-4-carboxilic acid
2,4a,7-Trihydroxy-1-methyl-8-methylenegibb-3-ene-1,10-dicarboxylic acid 1,4a-lactone

Other Name:
GA_3
Gibberellin A_3
Gibberellin X

Trade Names:
Pro-Gibb
Berelex

CAS Number:
77-06-5

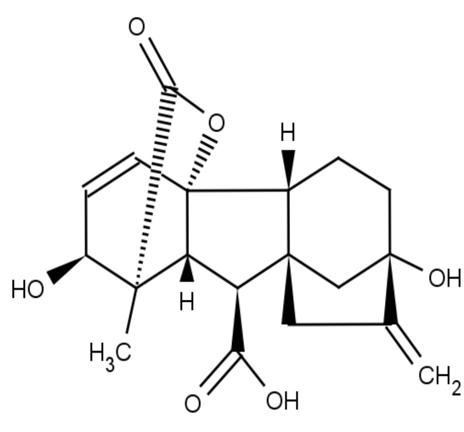
Other Codes:
EPA OPP Pesticide Chemical Code 043801
Caswell No. 467
EINECS 201-001-0
CIPAC No. 307

Characterization of Petitioned Substance

<u>Composition of the Substance</u>:

Gibberellic acid is a naturally occurring plant growth regulator within the family of plant hormones known as "gibberellins" (Machado and Soccol, 2008). It is produced by molds, fungi, and plants, though the main commercial source is the fungus *Gibberella fujikuroi* (HSDB, 2006). The compound gibberellic acid contains carbon, hydrogen, and oxygen, $C_{19}H_{22}O_6$. It is a diterpenoid acid, meaning that the basic structure is a combination of four isoprene units and a carboxylic acid structural unit (Harborne et al., 1999). The molecular structure is shown in Figure 1.

Figure 1. Molecular Structure of Gibberellic Acid



<u>Properties of the Substance</u>:

Gibberellic acid is a white to pale yellow crystalline powder with a molecular weight of 346.38 grams per mole (U.S. Pharmacopeia, 2010). It is soluble in water (ChemIDplus Lite, 2011) and very soluble in ethanol, methanol, and acetone (HSDB, 2006). Table 1 provides a list of physicochemical property values for gibberellic acid.

Table 1. Physicochemical Properties of Gibberellic Acid

Physical or Chemical Property	Value
Physical State	solid [+]
Appearance	white to yellow, fine powder [^]
Odor	Odorless [+]
Molecular Weight	346.38 g/mole [^]
Melting Point	234 °C [*^]
Solubility in Water	5 g/L (25 °C) [*]
Vapor Pressure	2.06E-13 mm Hg at 25 °C [*]
Henry's Law Constant	1.58E-15 atm-m^3/mole [*]
Density	600 mg/mL [+]
pKa Dissociation Constant	4 [*]
log Kow (octanol-water partition coefficient)	0.24 [*]
pH (of 5% solution)	4.0 [+]
bioconcentration factor (BCF)	3 [+]

[*] source: ChemID Plus Lite, 2011
[^] source; U.S. Pharmacopeia, 2010
[+] source: HSDB, 2006

Specific Uses of the Substance:

Gibberellic acid is used as a food additive, biopesticide, and plant growth regulator. The petitioned use is as a plant growth regulator.

Food Additive

Gibberellic acid is used as a food additive only in the malting of barley (21 CFR 172.725). It was first used in commercial brewing in the UK in 1959, and by 1973 was used in over 70% of all malt produced there (Hornsey, 2003). Gibberellic acid was not used by commercial brewers in the U.S. until the late 1960s (Hornsey, 2003), and its use reportedly remains uncommon as of 2003 (Bamforth, 2003).

Biopesticide

A biopesticide is a pest-controlling substance that is derived from natural materials (U.S. EPA, 2011a). As a biopesticide, gibberellic acid can improve resistance to fruit flies in citrus crops, such as grapefruits and oranges (Greany et al., 1991). Gibberellic acid delays the fruit peel senescence, meaning that the peel remains thick, hard, and oily, without delaying internal fruit development, theoretically making it more difficult for fruit flies to infest the fruit by laying eggs in the rinds (Birke et al., 2006). Giberrellic acid has been shown to be effective against Caribbean fruit flies and Mediterranean fruit flies when used pre-harvest on grapefruit and oranges (Greany et al., 1991), but not against Mexican fruit flies (Birke et al., 2006).

Gibberellic acid also is used to counteract the effects of fungal diseases by controlling the ripening process. It was originally registered in the U.S. in 1947 to counteract fruit russet fungus in apples (U.S. EPA, 1995). The petitioner's justification states that banana crops can be affected by fungal diseases such as Black Sigatoka during transport to market (Bujor, 2010). Black Sigatoka, which is caused by *Mycosphaerella fijiensis,* affects fruit ripening and can significantly decrease the economic value of a plantation (Jones, 2003).

Plant Growth Regulator

The main use of gibberellic acid, and the petitioned use, is as a plant growth regulator. Application of exogenous gibberellic acid to a plant will result in the same biological response as would happen if endogenous gibberellic acid was released from inside the plant cells (see "Action of the Substance") (Gent and McAvoy, 2000).

Gibberellic acid is widely used in commercial fruit production, to control fruit ripening, increase fruit size at harvest, and improve fruit's appearance in order to produce a more desirable, shippable product (Lindhot et al., 2008). It is most commonly used on grapes, citrus, and apple crops, but is also commonly used on pears, strawberries, blueberries, lettuce, artichokes, potatoes, rhubarb, and cherries (HSDB, 2006). Gibberellic acid is applied at different points during crop growth to achieve a variety of purposes, including but not limited to: increasing seed production, initiating flowering, quickening or delaying maturity of the fruit, increasing fruit yield, maintaining fruit's firmness as it ripens internally, and enlarging fruit size (HSDB, 2006). For example, gibberellic acid sprayed on navel oranges pre-harvest can maintain a sturdy rind composition as the fruit ages and delay rind coloration from green to orange without delaying internal fruit maturity (Lindhot et al., 2008). This allows the farmer to postpone harvesting until fruit have grown bigger and juicier, which is more desirable on the market. See "Action of the Substance," below, for more information about how gibberellic acid can effect growth and development of different crops.

In addition to pre-harvest treatments to control growth rates, gibberellic acid can be applied post-harvest to control fruit ripening and maintain quality through packaging, long-distance shipping, and shelf-life (Lindhot et al., 2008; Osman and Abu-Goukh, 2008). For example, when used on navel oranges, gibberellic acid increases rind firmness for easier packaging and shipping (Lindhot et al., 2008). Similarly, when used on bananas, gibberellic acid will delay fruit softening and color development, allowing for longer shipment time (i.e., further shipment distances) before bananas are considered "ripe" (Osman and Abu-Goukh, 2008).

The petitioner stated that the intended use for which the petition was submitted was "post-harvest on banana to prevent early ripeness" as well as post-harvest on citrus and pineapples to delay degradation and maintain freshness, thereby increasing shelf life (Bujor, 2010, 2011).[1] The petitioner indicated that shipping fruit to the U.S. from the tropics, where most organic bananas are grown, can take 15 to 21 days. Conventional, non-organic bananas are often treated with gibberellic acid, which keeps the bananas green for 5 or 6 more days and allows them to "resist the shipping time without ripening." Similarly, the petitioner states that gibberellic acid increases the shelf life of citrus and pineapple (Bujor, 2011). One study reports that post-harvest treatment of pineapples with a combination of gibberellic acid and naphthaleneacetic acid (NNA) extends the storage life from 12 – 15 days to 41 days (Quibo et al., 1997).

<u>Approved Legal Uses of the Substance:</u>

EPA pesticide regulations control the preharvest and postharvest use of substances such as gibberellic acid on raw agricultural commodities, whereas FDA regulations control the use of substances in processed foods.

In accordance with Section 4(g)(2)(A) of the Federal Insecticide Fungicide and Rodenticide Act (FIFRA), EPA performed a reregistration review in 1995 of pesticide products containing gibberellic acid as an active ingredient. , Based on this review, EPA supported the reregistration of all products containing gibberellic acid (U.S. EPA, 1995; HSDB, 2006). EPA has subsequently approved amendments to product registrations

[1] The U.S. EPA's 1995 Reregistration Eligibility Decision (RED) for gibberellic acid did not specifically identify post-harvest application on bananas as an eligible use (U.S. EPA, 1995). However, the petitioner received approval from EPA in June 2011 to amend its product label to include use on bananas and other crops (U.S. EPA, 2011b).

that allow additional uses. For example, in June 2011, EPA approved amendments to Registration No. 73 049-1 to add new uses for coffee, banana/plantain, pineapple, wheat, barley and oats (U.S. EPA, 2011b).

EPA's regulation of pesticides includes the establishment of limits, called "tolerances," on the amount of pesticides that may remain in or on foods marketed in the U.S. Gibberellic acid is exempt from the requirement of a tolerance for residues when it is used as a plant regulator in or on all food commodities, when it is applied to plants, seeds, or cuttings and on food commodities after harvest. It has been exempt from the tolerance requirements since June 1999 (40 CFR 180.1098).

Under FDA regulation 21 CFR 172.725, gibberellic acid is allowed for use as a food additive to be used in the malting of barley, provided that it meets the specifications of the regulation regarding purity and production source.

Action of the Substance:

Gibberellic acid is a naturally occurring plant growth hormone, within a family of plant growth hormones known as gibberellins (Mochado and Soccol, 2008). Gibberellins are biochemically active at concentrations as low as 0.001 ug/mL (Isaac, 1992).

When gibberellic acid naturally occurs in plants, it plays a role in regulating growth by inducing intermodal extension through promotion of cell elongation, reversing molecular signals for dwarfism, maintaining active cell division, and maintaining apical dominance (Isaac, 1992). Gibberellic acid also induces production and release of enzymes for starch synthesis and cell wall synthesis (Isaac, 1992).

Gibberellins, including gibberellic acid, initiate critical stages of plant growth and development in plants through triggering destruction of other cellular regulatory proteins, mainly those known as DELLA proteins (Arriizumi and Steber, 2006). DELLA proteins are transcription factors that exist in cells to maintain growth repression, i.e., the presence of DELLA proteins maintains a steady state of non-growth or non-development in the plant. The production of gibberellins in the plant (or, likewise, the artificial application of gibberellic acid to a plant) initiates a cellular pathway that results in degradation of DELLA proteins (Arrizumi and Steber, 2006). It is hypothesized that gibberellic acid acts by de-repressing genes that contribute to mRNA synthesis, which subsequently leads to synthesis of enzymes involved in starch and cell wall synthesis (Isaac, 1992). Application of exogenous gibberellic acid to a plant will result in the same biological response as would happen if endogenous gibberellic acid was released from inside the plant cells, and can even reverse the biochemical effects of endogenous gibberellin biosynthesis inhibitors, thereby inducing the plant's natural production of gibberellic acid (Gent and McAvoy, 2000).

A plant growth regulator is defined by EPA as "any substance or mixture of substances intended, through physiological action, for accelerating or retarding the rate of growth or rate of maturation, or for otherwise altering the behavior of plants or the produce thereof" (Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), section 2(v)). When gibberellic acid is applied to crops as a growth regulator, the purpose is to promote cell elongation resulting in larger fruits, eliminate dormancy of seeds and shorten germination, and/or affect flowering, sex expression, enzyme induction, and leaf and fruit senescence (Mochado and Soccol, 2008). For example, gibberellic acid sprayed on navel oranges when the fruit are 30 to 50 mm in size can reduce the occurrence of albedo breakdown (i.e., when rind tissue creases, cracks, and condenses, making the orange look unpleasant to consumers despite no effect to taste, nutrition, or shelf life of the fruit) (Lindhot et al., 2008). When used later on in fruit development, gibberellic acid will delay the coloration of the orange without delaying the internal fruit maturity, allowing the farmer to delay harvesting until the fruit grows larger (Lindhot et al., 2008). Similarly, gibberellic acid application will delay rind coloration in grapefruits and limes, delay maturity of lemons (Jackson and Looney, 1999), and delay ripening in bananas and tomatoes (Tingwa and Young, 1975). Application of gibberellic acid will increase fruit size in grapes (including Sultana, Black Corinth, and Delaware table grapes) when applied after full bloom. If applied before full bloom, it will induce seedlessness (Jackson and Looney, 1999). Used on sweet cherries, gibberellic acid delays fruit coloring, increases fruit size, and improves postharvest quality. It has been known to increase fruit set in pears after flowers were damaged by frost (Jackson and

Looney, 1999). Gibberellic acid slows ripening and senescence of fruits like tomatoes, bananas, and mangoes by delaying chlorophyll degradation and reducing sugar accumulation (Tingwa and Young, 1975; Osman and Abu-Goukh, 2008).

When gibberellic acid is used in beer brewing, it causes the barley grain to germinate faster by stimulating production of lytic enzymes that hydrolyse the starches in the endosperm of the grain seed (Hornsey, 2003). Gibberellic acid is naturally released by barley grains to stimulate production of lytic enzymes that control germination, but artificial application during the commercial brewing process is beneficial because it not only hastens the process, but also reduces malting loss, increases yield, and improves quality (Hornsey, 2003).

Combinations of the Substance:

When gibberellic acid is used in handling as the active ingredient in a pesticide and/or growth regulator, it is combined in formulation with other non-active ingredients. Non-active or "inert" ingredients that are allowed for use in pesticide formulations are identified by EPA List 4. To be used in organic crop production, the inert ingredients must be either considered natural or included on the National List of Allowed and Prohibited Substances (hereafter referred to as the National List). The National List states that substances classified as inert ingredients by EPA List 4 may be used for organic crop and livestock production, when used in combination with active ingredients that are nonsynthetic or synthetic but allowed by the National List (7 CFR 205.601(m)(1)). EPA List 4 inert ingredients are not included on the National List for organic handling/processing.

There are a number of anti-gibberellin growth retardants that are used in floriculture and ornamental horticulture to suppress growth and enhance flowering, and in agriculture to control shoot elongation, enhance flowering, and improve fruit quality (Jackson and Looney, 1999). These substances are often used in combination with gibberellic acid to control timing of plant growth. Anti-gibberellin growth retardants include daminozide, paciobutrazol, prohexidione-Ca, cycocel (Jackson and Looney, 1999). These anti-gibberellin growth regulators are not identified on the National List, and are not allowed for use in organic production.

Status

Historic Use:

Gibberellic acid was discovered in 1926, by a Japanese scientist named Kurosawa who was studying "foolish seedling" disease in rice crops (Hornsey, 2003). Kurosawa noted that the fungus *Gibberella fujikuroi* was infecting the diseased rice by secreting a substance that caused abnormal growth, and by 1939 a mixture of gibberellins had been isolated from the fungus and identified as the causative agent (Hornsey, 2003). Pure gibberellic acid was first isolated from the fungus in 1954 (Hornsey, 2003; Arteca, 1996). During the 1950s, extensive scientific research determined that similar substances existed in higher plants, and by 1991 it was determined that gibberellic acid was ubiquitous in plants. They are now considered to be widespread among angiosperms, gymnosperms, ferns, algae, fungi, and bacteria (Arteca, 1996). A fermentation process for mass production of gibberellins, including gibberellic acid, was developed in 1955 by scientists in the United States (Brueckner et al., 1989).

OFPA, USDA Final Rule:

Gibberellic acid is currently classified as a nonsynthetic substance and is allowed for use in organic production under NOP Rule Section 205.105. Gibberellic acid was previously reviewed by the National Organic Standards Board (NOSB) in September 1996 for use in organic crop production. It was determined to be nonsynthetic and was not prohibited for use in organic production, provided that it is produced from fermentation of non-genetically engineered organisms (NOSB, 1996). It has not been previously reviewed for use in organic handling.

International

Gibberellic acid is permitted for use by the Canadian organic standards, according to the most recent June 2011 amendment of the Canadian Organic Production Systems Permitted Substances Lists. It is included under Section 4, Permitted Substances List for Crop Production, Section 4.3 Crop Production Aids and Materials, allowed for use provided that it is made from a fermentation process and that process does not use genetically engineered organisms (CGSB, 2011). Gibberellic acid is not included under Section 6, Permitted Substances for Processing.

Gibberellic acid is not mentioned within the International Federation of Organic Agriculture Movements (IFOAM) Norms for Organic Production and Processing (IFOAM, 2006), the Japanese Agricultural Standard for Organic Processed Plants (Japanese MAFF, 2006), the East African Organic Product Standard (East African Community, 2007), or the Pacific Organic Standard (Secretariat of the Pacific Community, 2008).

Both the Codex Alimentarius Commission of the Joint FOA/WHO Food Standards Programme and the European Economic Community (EEC) Council Regulation does not specify regulation on gibberellic acid in organic production (Codex Alimentarius Commission, 2001; Commission of the European Communities, 2008).

Evaluation Questions for Substances to be used in Organic Handling

Evaluation Question #1: 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)).

Commercial-scale production of gibberellic acid is accomplished through a process involving submerged fermentation (SmF) techniques, usually with the *Gibberella fujikuoi* fungus[2] (Brueckner et al., 1989; Machado and Soccol, 2008). When the fungi are deprived of nitrogen, secondary metabolism is triggered resulting in biosynthesis of gibberellins, predominantly gibberellic acid (Machado and Soccol, 2008). A carbon-based substrate is used to feed carbon into the system, and as long as a sufficient carbon concentration remains gibberellin synthesis will continue (Rodrigues et al., 2009). Centrifugation or microfiltration is then used to separate the microbial cells from the fermentation broth (Machado and Soccol, 2008; Brueckner et al., 1989). Next, the gibberellins are recovered through adsorption onto activated charcoal, use of an ion exchange resin, or use of a liquid-liquid extraction process and then purified using methanol, acetone, ammonia/ammonium salts, and/or ethyl acetate (Brueckner et al., 1989). Specific details on the recovery and purification processes are generally not published, but rather kept as confidential business information by each manufacturing company (Brueckner et al., 1989).

In submerged fermentation, biosynthesis of gibberellins is stimulated in the presence of carbon and nitrogen, and the ratio of carbon to nitrogen is very important for determining how long production lasts and how much gibberellin is produced (Brueckner et al., 1989). Biosynthesis at reasonably high levels begins once nitrogen is exhausted from the system; however, production is higher if the initial nitrogen concentration is higher (Brueckner et al., 1989). In addition to nitrogen that is present naturally in the system, nitrogen can be added via addition of substances like ammonium sulfate, ammonium chloride, glycine, or ammonium tartrate (Brueckner et al., 1989). Another important factor of the fermentation system is the pH, which can influence the relative concentrations of gibberellins that are produced (Machado and Soccol, 2008; Brueckner et al., 1989). For example, gibberellic acid (GA3) is the most common

[2] *Gibberella fujikuroi* is also referred to in the literature as *Fusarium moniliforme* or *Fusarium fujikuroi*, depending on the stage of sexual reproduction that the fungi are in.

normal end-product of fermentation using *Gibberella fujikuroi* (Brueckner et al., 1989). While a pH of 3.5 to 5.8 will provide optimum concentrations of gibberellic acid production (Machado and Soccol, 2008), a higher pH (e.g., pH 7) will result in relative less gibberellic acid and more GA_4, GA_7, GA_9, GA_{12}, GA_{14} and GA_{16} . (Brueckner et al., 1989).

Figure 2 describes the submerged fermentation process in more detail.

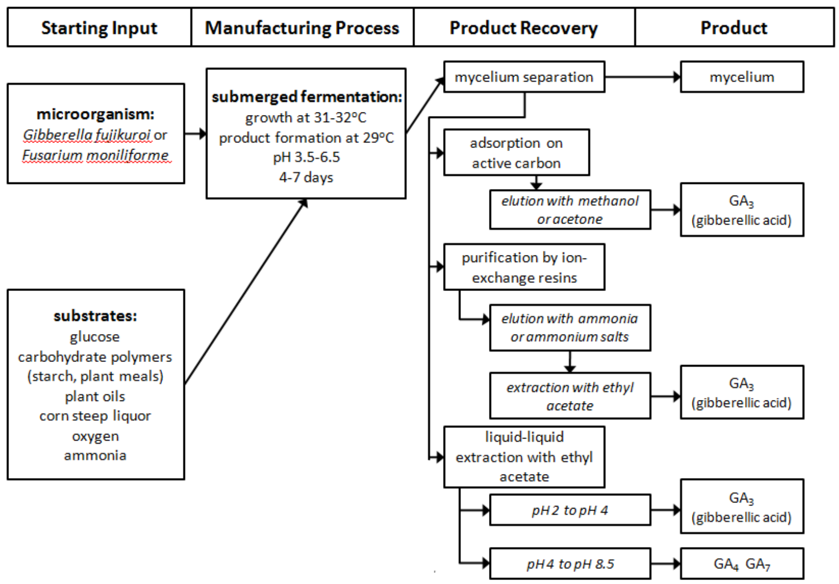


Figure 2. Submerged Fermentation Process
(Modified from: Brueckner et al., 1989)

The yield from SmF has remained very low, despite advances in technology (Machado and Soccol, 2008). Additionally, SmF has a high baseline cost: the centrifugation and filtration steps to separate the mycelium cells from the fermentation broth accounts for 48 to 76% of the manufacturing costs regardless of yield (Machado and Soccol, 2008).

As the cost of the SmF approach is high and the yield is low, studies have recently been performed on the feasibility of nonconventional methods such as solid state fermentation (SSF) (Machado and Soccol, 2008; Rodrigues et al., 2009).

SSF is a process defined by growth of microorganisms on moist solid materials in the absence of free water, in which a solid natural substrate is used as a carbon source or an inert substrate is used for solid support (Panday et al., 2008). SSF has been in use for food production since ancient history, as it was the process used for making bread in ancient Egypt and soy sauce by the Buddhists in the 7th Century (Panday et al., 2008). However, it was not until the 20th century that SSF was used to produce enzymes, organic acids, or secondary metabolites (Panday et al., 2008). Similar to SmF, the ratio of carbon to nitrogen in the system plays a key role in sustaining biosynthesis and the total amount of gibberellins produced (Rodriques et al., 2009). The choice of substrate, therefore, plays a key role in determining the productivity and economic feasibility of the SSF system. Table 4 presents research findings that illustrate how different substrates produce different amounts of gibberellic acid.

SSF has long been recognized as a higher-yield method than SmF for gibberellic acid production, however issues with refining and standardizing the process for consistency of yield and cost have kept it from being used on a wide scale or replacing SmF (Brueckner et al., 1989)—as the data in Table 4 demonstrate. Research has focused on determining the most efficient natural substrates for SSF, among a variety of different agricultural products and wastes (Barrios-Gonzalez and Mejia, 2008). Recently, techniques have been developed for SSF that increase yield of gibberellic acid by nearly 10-fold (Machado and Soccol, 2008). In addition to SSF systems using different types of substrates, researchers have introduced simplified model systems that involve the use of membrane filters (Barrios-Gonzalez and Mejia, 2008). The filters physically separate the fungus from the substrate so that it cannot grow into the substrate, which allows for complete biomass recovery from the substrate and subsequently higher yields (Rahardjo et al., 2004). These membranes introduce an artificial step to the biosynthesis and manufacture process, as they are made from synthetic materials like polycarbonate and result in changes to the metabolism and kinetics of the biosynthesis process (Rahardjo et al., 2004). No information was found to indicate how much commercial gibberellic acid is produced through SSF with natural substrates versus inert substrates, or with the use of a membrane filter.

Table 2. Maximum Productivity of SSF Systems
Using *Gibberella fujikuroi* and a Variety of Natural Substrates

Substrate	Bioreactor	Production	Reference
Glucose and glicina	6 L stirred fermentor	0.520 g/L	Hollmann et al., 1995
Glucose and rice meal	500 mL erlenmeyer flask	2.862 g/L	Escamilla et al., 2000
"	250 mL Erlenmeyer flask	1 g/L	Shukla et al., 2005
Wheat meal	50 L pilot-scale reactor	3 g/kg	Bandelier et al., 1997
Wheat meal and soluble starch	Glass columns	5 g/kg	Corona et al., 2005
Coffee husk	250 mL Erlenmeyer flask	3.3 g/kg	Rodrigues et al., 2009
Cassava bagasse	250 mL Erlenmeyer flask	1.1 g/kg	Rodrigues et al., 2009
Coffee husk and cassava bagasse	250 mL Erlenmeyer flask	0.493 g/kg	Machado et al., 2002
"	250 mL Erlenmeyer flask	3.0 g/kg	Rodrigues et al., 2009
Citric pulp	250 mL Erlenmeyer flask	5.7 g/kg	Rodrigues et al., 2009
Soy bran	250 mL Erlenmeyer flask	3.8 g/kg	Rodrigues et al., 2009

While plants or fungi other than *Gibberella fujikuoi* can be induced to biosynthesize gibberellins through the same techniques described above, the specific type of gibberellin and relative amount of each type within a mixture vary based on the species and even genetic constitution of the individual strain (Brueckner et al., 1989). For example, *Sphaceloma manihoticola* produces mainly GA_4, and, as previously stated, *Gibberella fujikuoi* produces mainly gibberellic acid (Brueckner et al., 1989). Further, different strains of *Gibberella fujikuoi* will produce different levels of gibberellic acid—one recent study showed that gibberellic acid production using soy bran as a substrate was 0 mg/kg for three *Gibberella fujikuoi* strains, but was over 3 mg/kg for two other strains (Rodrigues, 2009). Recently, scientists have characterized and cloned the genes associated with gibberellin biosynthesis in both *Fusarium fujikuoi* and the plant *Aribidopsis thaliana*, and have made progress in understanding regulatory mechanisms behind gibberellin biosynthesis, such as nitrogen metabolite repression (Tudzynski, 2005). This has allowed for research that could create improved-yield strains, such as gene cloning and amplification, construction of knock-out mutants, and controlled molecular biosynthesis regulation (Tudzynski, 2005).

No information was found to indicate the relative levels of commercial gibberellic acid production using SmF versus SSF techniques, using the conventional *Gibberella fujikuoi* versus other species, or using genetically modified strains of *Gibberella fujikuoi* versus non-modified strains.

After more than two decades of scientific attempts at synthesizing gibberellic acid, scientists were finally able to do so in 1982 (Corey and Munroe, 1982; as cited in Goldsmith, 1992; Corey, 1990). Since then, gibberellic acid has been produced synthetically in laboratory settings following a number of different schemes (Goldsmith, 1992). No information was found to indicate that laboratory synthesis of gibberellic acid is used for industrial/commercial production.

Evaluation Question #2: Is the substance synthetic? 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)).

Gibberellic acid may be isolated either through a nonsynthetic biosynthesis process or through chemical synthesis. When gibberellic acid was reviewed by the NOSB in September 1996 for use in organic crop production, it was determined to be nonsynthetic when produced from fermentation of non-genetically engineered organisms (NOSB, 1996).

Gibberellic acid is a naturally occurring plant growth regulator created through a biological process of secondary metabolite synthesis in fungi, bacteria, and higher plants (Machado and Soccol, 2008). In developing seeds and actively growing young plant shoots, gibberellins are produced through the mevalonic acid pathway (Arteca, 1996). Industrially, gibberellic acid is produced through a fermentation process that induces biosynthesis through manipulating the availability of carbon and nitrogen in a bioreactor (Rodrigues et al., 2009; Machado and Soccol, 2008; Brueckner et al., 1989).

When gibberellic acid is synthesized in a laboratory setting, it is formulated by a process that uses oxidation-reduction, esterification, thermolysis, and other chemical reactions to create changes in the chemical structure of the molecule (Goldsmith, 1992). Gibberellic acid produced this way is considered synthetic.

Evaluation Question #3: Provide a list of non-synthetic or natural source(s) of the petitioned substance (7 CFR § 205.600 (b) (1)).

Gibberellic acid is a naturally occurring plant growth hormone (Mochado and Soccol, 2008). Commercially available gibberellic acid is biosynthesized from natural sources in a fermentation process. Sources of gibberellic acid include fungi, bacteria, and higher plants (Machado and Soccol, 2008); the fungus *Gibberella fujikuroi* is currently used in industrial production (Rodrigues et al., 2009).

Evaluation Question #4: Specify whether the petitioned substance is categorized as generally recognized as safe (GRAS) when used according to FDA's good manufacturing practices (7 CFR § 205.600 (b)(5)). If not categorized as GRAS, describe the regulatory status. What is the technical function of the substance?

Gibberellic acid is not categorized as GRAS under FDA regulations at 21 CFR Parts 182, 184, or 186. FDA does regulate the use of gibberellic acid as a food additive in the malting of barley (21 CFR 172.725).

EPA pesticide regulatations govern the the use of gibberellic acid as a plant growth regulator. Gibberellic acid is exempt from section 408 of the FFDCA, 21 U.S.C. 346a(e) (as amended by the Food Quality Protection Act of 1996) requirement of a tolerance for residues, when it is used as a plant regulator in or on all food commodities when it is applied to plants, seeds, or cuttings and on all food commodities after harvest, as of 40 CFR Part 180.1098 (June, 1999). Gibberellic Acid was granted this exemption because of a non-toxic mode of action and low toxicity profile, combined with low application rates (64 FR 31501).

Evaluation Question #5: Describe whether the primary function/purpose of the petitioned substance is a preservative. If so, provide a detailed description of its mechanism as a preservative (7 CFR § 205.600 (b)(4)).

The main use, and the petitioned use, of gibberellic acid is as a growth regulator to control fruit ripening and increase fruit size and improve fruit's appearance in order to produce a more desirable, shippable product (Lindhot et al., 2008). The petitioner specifically mentions post-harvest use on bananas, pineapple, and citrus "to prevent early ripeness," "delay the degradation" of the fruit, and "maintain the freshness…after harvesting" (Bujor, 2010; 2011). Used in this way, gibberellic acid acts as a preservative of raw agricultural commodities post-harvest, and not as a preservative in processed food.

A secondary, non-petitioned use of gibberellic acid is as a biopesticide, for example to control infestation of fruit flies (Greany et al., 1991; Birke et al., 2006). If gibberellic acid is applied for this purpose, it is not considered a preservative.

<u>Evaluation Question #6:</u> Describe whether the petitioned substance will be used primarily to recreate or improve flavors, colors, textures, or nutritive values lost in processing (except when required by law) and how the substance recreates or improves any of these food/feed characteristics (7 CFR § 205.600 (b)(4)).

No information was found to suggest that gibberellic acid is used to recreate or improve flavors, colors, textures, or nutritive value lost in processing. Gibberellic acid is used to maintain color and texture through packaging, shipping, and shelf-life, which would normally be lost as natural ripening and degradation of the fruit occurs (Lindhot et al., 2008; Osman and Abu-Goukh, 2008). This use is not restorative.

<u>Evaluation Question #7</u>*:* Describe any effect or potential effect on the nutritional quality of the food or feed when the petitioned substance is used (7 CFR § 205.600 (b)(3)).

When gibberellic acid is used pre-harvest on oranges or other citrus fruits, it can decrease flavonoids and polyphenols in the juice of fruits treated in either the green or colored stages, as well as decreases carotenoids in the juice of fruits treated when they are in the green stage (Sandhu and Minhas, 2006). Flavonoids (such as kaempferol, and isoflavones) and polyphenols (such as tannins) are important micronutrients that serve antioxidant roles, protecting the body from cell and tissue damage that could occur due to free radical mediated oxidation (Fuhrman and Aviram, 2002). Carotenoids (such as beta-carotene and lycopene) are micronutrients that serve as a source of vitamin A and an antioxidant defense, and play a preventative role in cancer, cardiovascular disease, macular degeneration (which leads to blindness), and age-related illnesses (Sommerburg et al., 2002). No information was found indicating the extent of reduction in antioxidants like flavonoids, polyphenols, and carotenoids across various fruit types as a result of pre-harvest gibberellic acid treatment.

When applied post-harvest, gibberellic acid has been shown to reduce total sugar content in papayas over the course of the storage and ripening period, compared to papayas not treated with gibberellic acid (Ramakrishna et al., 2002). Similar researched showed that post-harvest treatment of tomatoes with 0.51 mg/kg, 0.71 mg/kg, and 0.68 mg/kg reduced total sugars present after 10 days of storage, and also slowed and reduced total carotenoids and lycopene levels compared to untreated controls (Pila, Gol, and Rao, 2010). Gibberellic acid has also been shown to interfere with the onset of starch degradation and interrupt the biosynthesis of sucrose in bananas, events that normally occur during the ripening processes (Rossetto et al., 2003). Gibberellic acid achieves the goal of delaying ripening in some fruits, such as bananas, by slowing the degradation of starch and biosynthesis of soluble sugars compared to a control. However, it does not stop degradation or biosynthesis altogether (Rossetto et al., 2003). Therefore, a fruit treated with gibberellic acid will have a varied nutritional make-up on a day-by-day basis compared to control, but there was no information found that indicated it does not eventually attain the same levels of "ripeness" and nutritional content as an untreated fruit if given more time prior to consumption.

<u>Evaluation Question #8:</u> List any reported residues of heavy metals or other contaminants in excess of FDA tolerances that are present or have been reported in the petitioned substance (7 CFR § 205.600 (b)(5)).

The specifications for gibberellic acid in the seventh edition of the "Food Chemicals Codex" include that it contain no more than 5 mg/kg lead (U.S. Pharmacopeia, 2010). No reports of excessive levels of heavy metals or other dangerous contaminants in gibberellic acid have been identified, and no substances listed on FDA's Action Levels for Poisonous or Deleterious Substances in Human Food have been reported as contaminants of concern for gibberellic acid.

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

The production and use of gibberellic acid as a growth regulator for commercial crops results in a direct release of gibberellic acid into the environment (HSDB, 2006). Based on chemical properties, gibberellic acid is assumed to have high motility in soil and is not expected to volatilize from either moist or dry soil surfaces (HSDB, 2006). If released into aquatic systems, gibberellic acid is not expected to adsorb to suspended sediment or volatilize, and the bioconcentration potential is low (HSDB, 2006). Additionally, gibberellic acid has been found to biodegrade by 85% after 5 days and by 90% after 10 days (Anderson et al., 1988). Gibberellic acid slowly hydrolyzes in water, and rapidly decomposes in the presence of heat or chlorine (Crop Protection Handbook, 2004).

No information was found to indicate that the industrial manufacturing process for production of gibberellic acid may be harmful to the environment or biodiversity.

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)).

No reports were found that indicated adverse effects upon human health from use of gibberellic acid. In 1995, the EPA reviewed the toxicity of gibberellic acid as part of a pesticide Reregistration Eligibility Decision (U.S. EPA, 1995). EPA determined that gibberellic acid has low acute toxicity, as studies of laboratory animals show no effects in tests for dermal sensitization or eye irritations and the concentration that caused lethality in 50% of test animals was high (2g/kg body weight in rabbits and 5 g/kg body weight in rats). Subchronic and developmental toxicity were also concluded to be low, and tests for mutagenicity were reported to be negative (U.S. EPA, 1995).

Evaluation Information #11: Provide a list of organic agricultural products that could be alternatives for the petitioned substance (7 CFR § 205.600 (b)(1)).

No organic agricultural products were identified that could serve as alternatives for the petitioned use of gibberellic acid.

Auxins are another group of plant growth regulators that, similar to gibberellic acid, can be used as bioregulators in agriculture to achieve effects such as larger fruit sizes and delayed ripening (Jackson and Looney, 1999; Tingwa and Young, 1975). Auxins can be synthetically produced or plant-derived. The common agriculturally used auxin 1-naphthalene acetic acid (NAA) is prohibited for use in organics as it is classified as a synthetic growth regulator (7 CFR § 205. 105(a)), whereas nonsynthetic indole acetic acid (IAA) is permitted in organic crop production, but it is not on the National List as a permitted non-organic ingredient for use in processing (7 CFR § 205. 105).

Ethylene is a naturally produced gaseous plant hormone that induces plant ripening, effectively counteracting the effects of gibberellic acid (Jackson and Looney, 1999). When used in industrial fruit production, ethylene gas is considered a synthetic nonagricultural substance. However ethylene is allowed for use in post-harvest ripening of tropical fruits, post-harvest degreening (i.e., color induction) of citrus, and pre-harvest induction of flowering in pineapples (7 CFR § 205.601(k) and 205.605(b)).

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