Indole-3-Butyric Acid (IBA)

Crop Production

Identification of Petitioned Substance

Chemical Name:

Indole-3-butyric acid.

Other Names:

IBA. 4-(3-Indolyl)butyric acid. 3-Indole butyric acid. Indolebutyric acid. IUPAC name: 4-(1H-indol-3-yl)butanoic acid.

Trade Names:

Hormodin. Seradix. Liba 10000. Jiffy Grow. Hormex.

CAS Number:

133-32-4.

Other Codes:

U.S. EPA PC Code: 046701. Registration Review Case #: 2330.

RTECS #: NL 5250000.

EINECS #: 205-101-5.

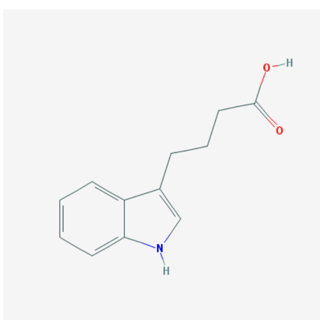
Characterization of Petitioned Substance

Composition of the Substance:

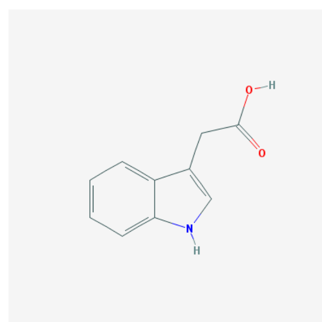
Indole-3-butyric acid (IBA) is a white to tan powder or crystalline solid with a slight characteristic odor. Formula: $C_{12}H_{13}NO_2$. Formula weight: 203.24. IBA may be viewed as a chemical compound with these two composing units: indole and butyric acid.

A similar compound is indole-3-acetic acid (IAA: CAS Number: 87-51-4. $C_{10}H_9NO_2$. Formula weight: 175.19). IAA may be viewed as a compound with these two composing units: indole and acetic acid.

Indole-3-butyric acid (IBA)



Indole-3-acetic acid (IAA)



Properties of the Substance:

IBA is stable at 2-8°C and should be stored in a cool place. The melting point of IBA is 121-125°C. IBA does not contain combustible liquids but decomposes to toxic fumes, such as NO_x, carbon monoxide, and carbon dioxide in fire. Its density is 0.60 g cm^{-3} at ambient temperature, and pH 3.54 for a 1% solution by weight dispersion in water.

IBA is practically insoluble in chloroform, but is soluble in alcohol, ether and acetone (Merck, 1989). The solubility of IBA in water is 250 mg L^{-1} (EPA, 2010). In order to make an aqueous IBA solution for purposes such as applying IBA to plant roots, IBA was dissolved with methanol, and this methanol solution was further diluted with water to make an aqueous IBA solution (Jemaa et al., 2011). The salt form of IBA, such as the sodium salt of indole-3-buryrate, is soluble in water (Fritz, 1962).

IBA is made into water-soluble and water-insoluble products (Kroin, 2008).

IBA decomposes when exposed to light (e.g. Nor Aini et al., 2009). However, specific information such as how fast IBA decomposes under various ambient conditions is still limited.

Specific Uses of the Substance:

IBA is a plant hormone in the "auxin"group. It is applied exogenously and used as a plant growth regulator (EPA, 1992). IBA exerts different effects on plant growth and development, e.g. regulating responses of plants against biotic and abiotic stresses (Tognetti et al., 2010), or increasing plant yield (Amin et al., 2006), but it is primarily implicated in adventitious root formation and widely used commercially for the induction of adventitious roots (Normanly et al., 1995; Ludwig-Muller, 2000; EPA, 2010).

Eight application methods are listed in EPA (1992). These methods may be separated to two groups, in terms of toxic effect and environmental consequence: point application and area application. Dipping plant cuttings in IBA dust, powder or solution and applying IBA dust, powder or solution to a plant cutting are point applications. Broadcasting IBA over turf, foliar spray, and adding IBA to a sprinkler system are area applications.

Approved Legal Uses of the Substance:

IBA is registered as a biochemical pesticide with U.S. EPA. Further details are given in the status of US EPA below.

Action of the Substance:

IBA is considered a plant hormone and is used primarily for the induction of adventitious roots. However, the mechanisms of IBA's role in plant growth and development are not well understood yet and are still under debate (Normanly et al., 1995; Ludwig-Muller, 2000; Ludwig-Muller, 2007; Pederson, 2007; Tognetti et al., 2010; Simon and Petrasek, 2011). Further details are given in Questions #11 and #12.

Status

U.S. Environmental Protection Agency

IBA is registered as a biochemical pesticide with the PC Code 046701. Historically, US EPA registered IBA as a synthetic hormone that is structurally related to the naturally-occurring plant hormone IAA (EPA, 1992). Recently, EPA became aware that IBA also occurs naturally in a variety of plant species (EPA, 2010) and would include this fact in its future documents about IBA (EPA, 1992; EPA, 2010).

IBA is registered with US EPA for use:

- to promote and accelerate root growth of plant clippings,
- to reduce transplant shock,
- to promote growth development of flowers and fruit, and
- to increase crop yields.

The content of 40 CFR 180.1158 "Auxins; exemption from the requirement of a tolerance" is quoted below:

> "An exemption from the requirement of a tolerance is established for residues of auxins (specifically: indole-3-acetic acid and indole-3-butyric acid) in or on all food commodities when used as plant regulators on plants, seeds, or cuttings and on all food commodities after harvest in accordance with good agricultural practices."

Sixty eight products containing IBA as their active ingredient are registered with US EPA for use as root stimulators, yield enhancers, fungicides, insecticides, and herbicides (PAN, 2011; EPA, 2010).

EPA (2010) provided the workplan for the registration review of IBA (Case 2330) in September 2010. The case will be developed. The final decision will be made in September, 2011, according to EPA's final work plan (EPA, 2010).

OMRI Status

OMRI evaluates materials for compliance with USDA NOP regulations, 7 CFR Part 205. A substance not allowed under the USDA NOP regulations is listed as "prohibited" in the OMRI Generic Materials List.

Plant hormones such as IBA and NAA are listed in the OMRI Generic Materials List (OMRI, 2009) as "Growth regulators for plants – Prohibited – Synthetic." Plant hormones such as gibberellic acid, IAA, and cytokinins are listed in the OMRI Generic Materials List (OMRI, 2009) as "Growth regulators for plants – Allowed with restrictions – Nonsynthetic." The OMRI list is updated recently in 2011 but the status of these substances is not changed.

International and other regulations:

IBA is an "Acute Health Hazard" and "Chronic Health Hazard" under Section 311/312 Hazard Classes of SARA Title III Rules (MSDS-IBA, 2007).

European Commission: IBA is listed in EINECS as 205-101-5. IBA is not classified in the Annex I of Directive 67/548/EEC, not listed in the Annex I of Regulation No 689/2008, not reported by EU Industry as an HPVC or LPVC, and is not listed in a priority list No 793/93. The IUCLID and OECD Chemical Data Sheets and Export Files information are not available for IBA. There is no entry in ESIS for IBA with respect to the Biocidal Products Directive (Directive 98/8/EC) information.

WHMIS (Canada): IBA is not controlled under WHMIS (Canada).

IBA is "no data", "not applicable", or "not listed" in numerous "Federal Regulations", "Other Federal Regulations", "State Regulations," and "International Regulations" (PAN, 2011; MSDS-IBA, 2004; MSDS-IBA, 2007; MSDS-IBA, 2009; MSDS-IBA, 2010).

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

Evaluation Question #1: A) Does the substance contain an active ingredient in either 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 warps and seals, insect traps, sticky barriers, row covers, and equipment cleansers? (B) Does the substance contain synthetic inert ingredients that are not classified by the EPA as inerts of toxicological concern (i.e., EPA List 4 inerts)? (7 U.S.C. § 6517(c)(1)(B)(i)). Does the synthetic substance contain inert ingredients which are not on EPA List 4, but are exempt from a requirement of a tolerance, per 40 CFR part 180?

IBA is petitioned to be used as a plant growth regulator for enhancing plant propagation from cuttings and for increasing plant yield and quality (Kroin, 2008).

A) IBA ($C_{12}H_{13}NO_2$) may be viewed as a substance with two composing units: indole (C_8H_7N) and butyric acid ($C_4H_8O_2$), in terms of its chemical composition. It is one molecule and does not consist of ingredients. IBA itself is not an ingredient in the substances listed in A. IBA is manufactured in industry, biosynthesized by natural plants, and produced by soil bacteria. However, IBA is considered and used as a plant hormone.

B) IBA itself is not listed in EPA List 4 Inert Ingredients. However, IBA is exempt from a requirement of a tolerance (40 CFR 180.1158).

Evaluation Question #2: 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))

The petitioner stated that the substance (IBA) is a technical grade synthesized substance from many commercial sources (Kroin, 2008).

IBA is manufactured and sold in the United States and in other countries (e.g. MSDS-IBA 2004; 2007; 2009; 2010). One hundred eighty six products containing IBA are available in the United States (PAN, 2011). Sixty-eight products are registered with US EPA (EPA, 2010). The PAN Pesticides Database (PAN) provides a list of 106 registered products (PAN, 2011). Specific manufacturing procedures are not available. The description of starting materials, production processes, and formulation processes for the product registered with US EPA is CBI (Confidential Business Information) (EPA, 2010).

Chemical Process

One example of industry-scale production is given in US Patent 3,051,723 (Fritz, 1962). Indole (CAS #: 120-72-9), γ-butyrolactone (CAS #: 96-48-0), and sodium hydroxide are mixed to react chemically at 245°C in a stainless steel container for 20 hours. The lactone ring is opened and the opened lactone is added to the C3 position of indole. The reaction product (butyrate and other chemicals) is dissolved in water. The un-reacted chemicals such as indole are extracted into isopropyl ether and separated. Dissolved indole-3-butyrate (salt) is acidified with hydrochloric acid and converted into IBA (acid). This IBA is collected as the precipitate since IBA dissolves very slightly in water.

Cohen and Schulze (1981) and Pederson (2007) followed the above U.S. Patent and produced IBA in a laboratory scale. Alternatively, IBA was produced chemically from ethyl cyclohexane-1-one-2-carboxylate (Jackson and Manske, 1930).

Naturally Occurring Biological Processes

Plant hormones are groups of substances which promote, inhibit, or modify the growth and development of plants. These substances are produced by plants, algae, fungi, and plant growth-promoting rhizobacteria (Costacurta and Vanderleyden, 1995) (Rhizobacteria are root-colonizing bacteria that form a symbiotic relationship with plants). For example, indole-3-acetic acid is one of the common plant hormones (Normanly et al., 1995), is biosynthesized in natural plants, and is produced by the cyanobacterium *Arthrospira platensis* strain MMG-9 (Mehboob et al., 2010), by *Rubrivivax benzoatilyticus* strain JA2 (Mujahid et al., 2011), and by several wild-type strains of *Ustilago maydis* (Martinez et al., 1997).

Similar to IAA, IBA is one chemical in the auxin group. IBA is found to be biosynthesized from IAA (Ludwig-Muller and Epstein, 1991; Ludwig-Muller and Hilgenberg, 1995; Ludwig-Muller et al., 1995). IBA, as well as other indole auxins of IAA, indole-3-carboxylic acid, and indole-3-propionic acid, was extracted from nicotiana (e.g. *N. glauca* and *N. langsdorffii*) (Bayer 1969) and other plants (Schneider et al., 1985; Ludwig-Muller, 2000; Ludwig-Muller and Cohen, 2002; Pederson, 2007). IBA, as well as IAA, was produced in culture medium by wild strain of bacterium *Azospirillum brasilense* (Fallik et al., 1989; Martinez-Morales et al., 2003).

The occurrence of IBA in natural plants is further discussed in Question #11.

<u>Evaluation Question #3:</u> 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))

As given in Question #2, IBA is manufactured worldwide. The petitioned substance is from many commercial sources (Kroin, 2008). Specific manufacturing processes are not available. However, the production of IBA from indole and γ-butyrolactone (Fritz, 1962) might be one of the prevalent processes.

The concentrations of IBA in natural plants are on the ng g^{-1} to sub μg g^{-1} level (Epstein and Ludwig-Muller, 1993; Ludwig-Muller and Cohen, 2002). The literature about extracting IBA from natural plants for commercial use is limited.

As given in Question #2, IBA is manufactured in industry, biosynthesized in natural plants, and produced by soil bacteria. IBA is a basic chemical compound. There has been no evidence to indicate that the chemical properties of IBA extracted from natural plants are different from the chemical properties of IBA manufactured in industry. Actually, the identification of extracted IBA from natural plants is based on the agreement of properties of extracted IBA with the properties of industry-manufactured IBA.

Manufactured IBA products unavoidably contain impurities such as by-products and un-reacted raw materials. IBA is manufactured and sold internationally. Detailed composition of each IBA product is not available. One product, registered with US EPA (EPA, 2010), contained the following components: 98.19-98.30% IBA, 0.96-1.06% 4-(indol-3-yl)-4′-[2-(butyric acid-4-yl)indole-3-yl]butyric acid, and 0.27-0.34% 4-[2-(butyric acid-4-yl)indole-3-yl]butyric acid.

The petitioned substance is technical grade, as claimed by the petitioner (Kroin, 2008).

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

The effect of IBA regulating plant growth was discovered in the 1930s (Cooper, 1935), but the natural presence of IBA in plants was confirmed in the 1990s (Schneider et al., 1985; Epstein and Ludwig-Muller, 1993) because of IBA's low concentrations in plants and lack of sensitive analytical instruments (Kende and Zeevaart, 1997; Ludwig-Muller and Cohen, 2002).

Schneider et al. (1985) confirmed the endogenous presence of IBA in pea seedling organs but failed to estimate quantitatively due to its low presence. The concurring IAA was the highest in the root (20 ng g^{-1} fresh weight) and lowest in the cotyledons (3 ng g^{-1}).

Some plant tissues contained 9 ng g^{-1} fresh weight of free IBA and 37 ng g^{-1} fresh weight of total IBA (Epstein and Ludwig-Muller, 1993). As a reference, the concurring fresh weight concentrations of free IAA and total IAA were 26 and 52 ng g^{-1}, respectively.

The concentration of IBA in *Tropaeolum majus* was 61 (root), 21 (hypocotyl), 16 (shoot), 11 (leaf stalk), 22 (older leaf), and 30 (young leaf) ng g^{-1} (fresh weight), respectively in different plant organs (Ludwig-Muller and Cohen, 2002). The concentration of IAA was also the highest in roots (119 ng g^{-1}) and became lower in other plant organs.

Free IBA and free IAA, extracted from *Arabidopsis thaliana* seedlings, were about 15 and 25 ng g^{-1}, respectively (Pederson, 2007). Pederson (2007) compiled a list of 11 plant species or organs from which IBA was extracted and identified.

The root surface of maize seedlings which were inoculated with *Azospirillum* increased significantly two weeks after sowing as compared to non-inoculated plants. Both IAA and IBA were found in the roots. IAA was found but IBA was not detected in the culture medium (Fallik et al., 1989). *Azospirillum brasilense* was grown in a culture medium. IAA and IBA were found in the culture medium (Martinez-Morales et al., 2003).

EPA stipulated that IBA does not persist in the environment (EPA, 1992; EPA, 2010).

<u>Evaluation Question #5:</u> Describe the toxicity, mode of action and breakdown products of the petitioned substance any known toxic or other adverse action of the substance and/or its breakdown products. (7 U.S.C. § 6518 (m) (2))

Relevant to Questions #5 - #10, the common amount used in various applications was from 0.5 mg L^{-1} to less than 1% (10,000 mg L^{-1}) in solution (e.g. Kroin, 2008; Amin et al., 2006; Amin et al., 2007; Ercisli et al., 2003; Karakurt et al., 2009; Nor Aini et al., 2009; EPA, 2010). "All currently registered end products formulated with IBA are applied in ultra-low quantities, up to 7 mg active ingredient/acre/crop season for the crop uses, and similar low applicator exposure for ornamental plant propagation," (EPA, 1992).

The primary application is to dip plant cuttings in IBA solution or IBA powder for inducing the adventitious root formation (Kronin, 2008). Foliar spray of IBA is used in enhancing crop yield (Amin et al., 2006).

IBA is being petitioned to be used as a plant growth promoter. "Low toxicity" was claimed by the petitioner (Kroin, 2008) for both the active ingredient (i.e. IBA) and possible breakdown products.

EPA evaluated the toxicity of IBA based on the following rational. IBA is similar in structure and function to naturally occurring IAA (actually, IBA, based on the recent literature, is also naturally occurring). IBA is metabolized to IAA in the human body. IBA has a non-toxic mode of action. EPA concluded that IBA has low acute toxicity with the exception that IBA is an eye irritant (EPA, 1992; EPA, 2010).

40 CFR 180.1158 exempted IBA from the requirement of a tolerance for residues of IBA in or on food commodities when used as plant regulators. "All generic toxicology data requirements have been waved for IBA," EPA (1992).

EPA stipulated that IBA does not persist in the environment (EPA, 1992; EPA, 2010).

Literature about the toxicity of IBA is limited.

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

As IBA is manufactured and sold internationally, there might be different manufacturing procedures. One example of industrial production is provided by Fritz (1962). As given in Question #2, indole, γ–butyrolactone, and sodium hydroxide are the primary chemical reagents for making butyrate at 245°C. Isopropyl ether and hydrochloric acid are other chemicals used to remove un-reacted chemicals or to convert butyric salt to butyric acid so that pure IBA is produced.

The petitioned substance is a technical grade chemical (Kroin, 2008). One product, registered with US EPA, contained > 98% IBA, ~1% 4(indol-3-yl)-4'-[2-(butyric acid-4-yl)indol-3-yl]butyric acid, and < 0.5% 4-[2-(butyric acid-4-yl)indol-3-yl]butyric acid (EPA, 2010).

IBA itself does not contain carcinogens or potential carcinogens listed by OSHA, IARC or NTP. It has a non-toxic mode of action (EPA, 1992; EPA, 2010). It does not explode in flame (PAN, 2011; MSDS-IBA: 2007, 2009 and 2010).

Indole (CAS #: 120-72-9), γ–butyrolactone (CAS #: 96-48-0), and sodium hydroxide (CAS #: 1310-73-2) are included in U.S. EPA "List of Inert Pesticide Ingredients – List 4B."

Isopropyl ether is listed as "UN1159 Flammable Liquid" by U.S. DOT. The health effects are listed in OSHA as "Irritation-Eye, Nose, Throat, Skin --- Mild (HE15)."

Hydrochloric acid is a common chemical in industry (HCl Facts, 2011).

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))

IBA potentially reacts with strong oxidizers (MSDS-IBA, 2007; MSDS-IBA, 2009 and MSDS-IBA, 2010).

IBA is used and found effective as growth promoter and rooting stimulator in various applications. The amount used for this purpose is from trace to minor, as given in Question #5. Plant hormones are not nutrients to plants but chemicals, even at trace to minor amounts, regulate plant growth. The stimulating effect of IBA is synergetic with other chemicals and bacteria (Drew et al., 1991; Christov and Koleva, 1995; Falasca et al., 2000; Takahashi et al., 2003; Ercisli et al., 2003; Amin et al., 2006; Amin et al., 2007; Karakurt et al., 2009; Agullo-Anton et al., 2011; Strader et al., 2011; Mori et al., 2011). Auxins such as IAA and IBA stimulated rooting in the first and the second phases of rooting but suppressed the rooting in the third phase (De Klerk, 2002). Therefore, an excessive usage of IBA might lead to some unfavorable consequences in plant growth. However, the literature about these potential unfavorable consequences is limited.

Dipping cuttings in IBA solution or powder and foliar spray of IBA are the primary means of applying IBA for plant growth and plant propagation. Foliar spray of IBA is used in enhancing rooting and crop yield (Samananda et al., 1972; Kroin, 1992; Blythe et al., 2004; Amin et al., 2006; Khandagale et al., 2009; Abdel, 2011). "Applicability to a wide range of crops has yet to be established. Examination of the variability in absorption and translocation of foliar-applied auxin to the site of root initiation may merit further study," Blythe et al. (2004). The used amount is limited, as given in Question #5. The petition (Kroin, 2008) claimed "no interactions" of IBA with other substances used in organic production. The literature about IBA's potentially detrimental chemical interaction with other substances used in organic crop or livestock production is scarce.

No data or evidence is listed in the PAN database about "terrestrial ecotoxicity" (PAN, 2011). Slight toxicity is listed towards fish in the category of "aquatic ecotoxicity" (PAN, 2011).

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, crops, and/or livestock. (7 U.S.C. § 6518 (m) (5))

IBA is biosynthesized in natural plants and produced by soil bacteria.

IBA is petitioned to be used as a plant hormone. The application amount is from "trace to minor", as given in Question #5. The literature about potential detrimental physiological effects is limited. Instead, indole derivatives including IBA possess fungicidal activity against some plant pathogenic fungi (Abdel-Aty, 2010).

<u>Evaluation Question #9:</u> Discuss and summarize findings on whether 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))

As given in Question #5, the used amount of IBA in various applications is trace to minor.

EPA (1992) listed a set of facts and reasonable assumptions about IBA's effect on organisms and environment: the applied amount of IBA is low; IBA is a plant hormone but not a toxicant or repellant; IBA is structurally and functionally similar to other natural auxins; and IBA might also occur naturally in plants.

"IBA has been shown to be practically nontoxic to avian species. … … IBA should not cause any adverse effects to avian wildlife," (EPA, 2010). IBA is not known to be phytotoxic. IBA should not cause any adverse effects to mammalian wildlife. EPA (2010) further discussed the toxicity of IBA to nontarget insects and to threatened species, and indicated that testing data may not necessarily be needed urgently.

IBA is shown to be slightly toxic to fish and aquatic invertebrates (EPA, 1992; EPA, 2010).

No data or evidences are listed in the PAN database about the harmful effect of IBA to the environment, except some slight toxicity towards fish (PAN, 2011).

<u>Evaluation Question #10:</u> 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))

EPA (2000) concluded that "this plant growth regulator poses no known risks to humans or the environment," and "in animals, indole-3-butyric acid is rapidly broken down to a closely related, harmless chemical that occurs naturally in living organisms."

IBA occurs naturally in plants and is produced by soil bacteria, as given in Questions #2 and #3. The usage of IBA is limited in amount and in location, as given in Question #5. "IBA is metabolized into IAA in the human body and IAA is a common metabolite in tryptophan metabolism in human," EPA (1992). 40 CFR 180.1158, given above in the "Status" section, exempts the residues of IBA (and IAA) in or on food commodities from the requirement of a tolerance.

IBA is "not listed", "not available", "no NTP studies", and "no" in the categories of "Acute Toxicity", "Cancer Information", "Endocrine Disruption", "Reproductive and Developmental Toxicity" and "Chemicals of Special Concern" in the PAN Pesticides Database (PAN, 2011).

EPA, in 1992, waived all toxicity data requirements for the pesticide registration of IBA because the general exposure and the dietary exposure to the products containing IBA were expected to be very low (EPA, 1992). EPA, based on recently available data, provided that IBA, the active ingredient in the registered products, is a Toxicity Category III or IV substance, in terms of "acute oral toxicity", "acute dermal toxicity", "acute inhalation toxicity" and "acute dermal irritation" (EPA, 2010). Two LD_{50} values are listed here as examples for evaluating IBA's toxicity: acute oral LD_{50} > 2,000 mg kg^{-1} (rat); and acute dermal LD_{50} > 2,000 mg kg^{-1} (rabbit).

IBA might be a Toxicity Category II material in terms of corneal involvement and/or irritation cleaning in 8-21 days (EPA, 2010).

The data requirements for other items are still waived: "90-day oral", "90-day dermal", "90-day inhalation" and "prenatal developmental" (EPA, 2010; 40 CFR 180.1158).

<u>Evaluation Question #11:</u> Describe all natural (non-synthetic) substances or products which may be used in place of a 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))

Plant Growth and Plant Hormones

Plants grow in the format of cell production in the meristems and ensuring elongation of these newly formed cells (Clark, 1997; Kerstetter and Hake, 1997; Schiefelbein et al., 1997). Plant hormones are not nutrients but are chemicals regulating the cell division and cell elongation, and thus play an important role in the growth and development of plants.

Five groups of chemicals are considered as "classical" plant hormones: gibberellins, cytokinins, abscisic acid (ABA), ethylene, and auxins (Kende and Zeevaart, 1997). However, more chemicals could be found as plant hormones with the development in analytical chemistry (Kende and Zeevaart, 1997; Ludwig-Muller and Cohen, 2002). For example, brassinosteroids are proposed as another group of plant hormones (Martinez-Morales et al., 2003). One group of plant hormones might actually consist of numerous chemicals. For example, gibberellins contained 112 identified chemicals (Kende and Zeevaart, 1997).

Plant hormones regulate the cell division and cell elongation in general, but each group of plant hormones further posses some dedicated functions (Pederson, 2007). For example, auxins stimulate root growth, gibberellins control flowering, and abscisic acid inhibits the effects of other hormones to reduce growth during times of plant stress (Schalau 2005; Whiting, 2010).

Auxins consist of chemicals such as IAA, IBA, indole-3-propionic acid (IPA), and naphthalene acetic acid (NAA) (Martinez-Morales et al., 2003; Pederson, 2007). IAA, IBA, phenyl acetic acid, and 4-chloro-IAA are found in natural plants. The concentrations of these hormones in natural plants are very low, at ng g^{-1} or sub µg g^{-1} level (Epstein and Ludwig-Muller, 1993; Pederson, 2007; Ludwig-Muller and Cohen, 2002). Several auxin components, such as IBA and 4-chloro-IAA, have to be at higher concentrations to exert similar functions as IAA (Normanly et al., 1995), but there are also evidences that IBA is more effective than IAA in inducing adventitious rooting (Nordstrom et al., 1991; Epstein and Ludwig-Muller, 1993; Ludwig-Muller, 2000; Ludwig-Muller et al., 2005; Jemaa et al., 2011).

The physiological roles of plant hormones are not well understood yet (Costacurta and Vanderleyden, 1995; Kende and Zeevaart, 1997). Recent research provides better understanding of the molecular mechanisms underlying the physiological role of auxin in plant development (Pederson, 2007; Simon and Petrasek, 2011).

Even though the physiological roles of plant hormones are not well understood, plant hormones, as a whole category of substances, are considered as essential (Sorefan et al., 2009; Strader et al., 2011; Simon and Petrasek, 2011) and practically used in various plant propagation and plant growth applications.

Plant hormones regulate plant growth not only directly, but also through the synergetic effects with other chemicals, carbohydrates (Takahashi et al., 2003; Agullo-Anton et al., 2011) and/or soil bacteria (Falasca et al., 2000; Ercisli et al., 2003; Amin et al., 2007; Karakurt et al., 2009; Agullo-Anton et al., 2011; Strader et al., 2011; Mori et al., 2011). In this sense, a great availability of different plant hormones might provide sufficient flexibility for different crop production (Simon and Petrasek, 2011).

Plant Propagation

"Plant propagation is the process of multiplying the numbers of a species, perpetuating a species, or maintaining the youthfulness of a plant," MG Manual (1998). Sexual propagation involves the floral parts of a plant and offers the possibility of evolving to new species. Asexual propagation uses a part of one parent plant, causes it to regenerate itself into a new plant, and offers the opportunity of keeping parent plant's genetic characteristics. The advantages and disadvantages of each propagation method have to be comprehensively evaluated based on specific scenarios, in terms of propagation efficiency (propagation quantity, operation easiness, operation cost, time involved, facility availability, etc), propagation quality (weather adaptation, transmission of certain diseases, etc), new species evaluation, preservation of parent plant's unique genetic characteristics, and other factors (e.g. Hilaire, 2003; Koyuncu and Senel, 2003; Ludwig-Muller et al., 2005).

Asexual propagation methods are cuttings, layering, division, and budding grafting (MG Manual, 1998). Cuttings involve rooting a severed piece of the parent plant. Evans and Blazich (1999) provided an introduction to plant propagation by stem cuttings. The plant propagation by stem cuttings is further described by Bir and Bilderback (2011). Seedlings, root cuttings and rhizome cuttings (WSU, 2011), in addition to stem cuttings, are other ways of plant propagation. Depending on which part of one parent plant is used, cuttings may be further identified as stem cuttings, root cuttings, and other seven more cuttings (MG Manual, 1998).

De novo root formation in nonroot plant organs, or adventitious root formation (ARF), is influenced by a combination of endogenous and environmental factors (Agullo-Anton et al., 2011). Particularly adventitious root formation is regulated by a group of chemicals called auxin, one group of plant hormones.

All Natural Substances Used in Place of IBA

IBA is being petitioned to be used as a plant growth regulator for enhancing plant propagation from cuttings and for increasing plant yield and quality (Kroin, 2008). As being contrasted to the overall pictures of plant propagation and plant hormones given above, IBA is one of numerous plant hormones. Propagation from cuttings is one of numerous plant propagation processes. Since the mechanisms of IBA in inducing adventitious rooting are not clearly understood, it might be difficult to assess what natural substances could be used to replace IBA in general. Furthermore, the concentrations of IBA in natural plants are very low, at ng g^{-1} level.

Currently, "European and North American regulations do not allow the use of synthetic products to obtain organic vegetative propagation materials," Centeno and Gomez-del-Campo (2008). Centeno and Gomez-del-Campo (2008) briefly introduced methods in plant propagation without using synthetic materials. Cuttings were enclosed with germinating seeds to stimulate root formation since germinating seeds contained natural auxins. Fungi produced auxins and contained proteins, carbohydrates, lipids, minerals, and vitamins. Algae also contained IAA, proteins, lipids and carbohydrates. Yeast extract was used for root growth in *Glehnia littoralis*. Mineral nutrients and vitamins were found improving the rooting of cuttings (Christov and Koleva, 1995).

Specifically, Centeno and Gomez-del-Campo (2008) confirmed that IBA was effective in promoting rooting of cuttings. The rooted cuttings of organic olive plants (*Olea europaea* L. cv. Cornicabra) were 47.2%, when IBA was used, compared to 5.5% rooted cuttings of control group. Meanwhile, Centeno and Gomez-del-Campo (2008) evaluated the effects of several alternative materials: ALG (alga *Fucus* extract), YEA (yeast powder *Saccharomyces cereviseae*), SEE (sunflower seeds), Sm-6 (Sm-6 Organico™, a mixture of *Ascophyllum nodosum, Fucus serratus, Laminaria hyoborea*, and *Laminaria digitata* algae dry extract, containing growth hormones and essential nutrients), and Terrabal (Terrabal Organico™, an extract of macerated cereal seeds, containing soluble proteins, amino acids, vitamins, nitrogen, phosphorus and potassium). Sm-6 and Terrabal are authorized in organic agriculture as a biostimulant for horticultural crops (Centeno and Gomez-del-Campo, 2008). The rooting percentages were ALG: 36.0%, YEA: 36.1%, SEE: 8.3, Sm-6: 58.0% and Terrabal: 56.0%, respectively. Based on further experimental results, Centeno and Gomez-del-Campo (2008) proposed that Terrabal Organico™ could be an alternative to IBA in the propagation of organic olive plants of cv. Cornicabra.

IAA and IBA

IAA and IBA are the two important components in the plant hormone group "auxins". IAA is the first auxin to be used to stimulate rooting of cuttings (Cooper, 1935) and is the main plant hormones in this auxin group (Kende and Zeevaart, 1997; Pederson, 2007; Simon and Petrasek, 2011). IAA is found in natural plants (Pederson, 2007). Maintaining the endogenous pool of IAA at an appropriate level among different plant organs is one of the mechanisms plant hormones regulate the growth of plants (Epstein and Lavee, 1984; Park et al., 2007; Ludwig-Muller, 2007; Jemaa et al., 2011).

IBA is also found to distribute in natural plants (Epstein and Ludwig-Muller, 1993; Pederson, 2007) and confirmed as an endogenous constituent of various plants (Ludwig-Muller, 2000). For example, "plant tissues contained 9 ng g^{-1} fresh weight of free IBA and 37 ng g^{-1} fresh weight of total IBA, compared to 26 ng g^{-1} and 52 ng g^{-1} fresh weight of free and total indole-3-acetic acid (IAA), respectively," Epstein and Ludwig-Muller (1993).

By using radioactive IBA, Epstein and Lavee (1984) confirmed that IBA was converted to IAA by cuttings of grapevine and olive. IBA can be converted to IAA and IAA can be a precursor to IBA (Bartel et al., 2001; Strader et al., 2011), as it is pictured in Fig. 1 of Ludwig-Muller (2011). IBA might be entirely synthesized from IAA (Simon and Petraske, 2011). It might serve as a more stable storage form of IAA. That is, IBA is converted back to IAA if plant IAA is depleted. A possible IAA-independent pathway for IBA biosynthesis has still to be provided (Simon Petraske, 2011).

Other auxin compounds in general are active only at higher concentrations than IAA and their role in growth remains largely unknown (Normanly et al., 1995; Pederson, 2007). In recent years, some of these other auxins such as IBA were found more effective in stimulating rooting than IAA during certain developmental stages or in certain plant species (Nordstrom et al., 1991; Epstein and Ludwig-Muller, 1993; Ludwig-Muller, 2000; Ludwig-Muller et al., 2005; Jemaa et al., 2011). IBA was used in recent researches for promoting root growth (Hilaire, 2003; Amin et al., 2007; Karakurt et al., 2009; Nor Aini et al., 2009; Jemaa et al., 2011).

The biosynthesis of IBA in plant might be controlled by external and internal factors/processes, for example, in maize (*Zea mays* L.) (Ludwig-Muller, 2000) and in *Arabidopsis thaliana* (Strader and Bartel, 2009; Tognetti et al., 2010). After microcuttings were processed for rooting, auxins such as IAA and IBA stimulated rooting in the first phase (dedifferentiation, 0-24 hrs) and the second phase (induction, 24-96 hrs), but actually inhibited rooting in the third phase (differentiation, from 96 hrs onwards) (De Klerk, 2002).

The degradation of IAA is affected by nutrient salts and by light. When both nutrient salts and light acted synergistically, 80% of the original IAA in one incubation solution degraded within seven days (Dunlap and Robacker, 1988). IBA is also degraded by light (e.g. Nor Aini et al., 2009). However, Nissen and Sutter (1990) found that IBA was significantly more stable than IAA to autoclaving. “The concentrations of IAA and IBA in autoclaved medium were reduced by 40% and 20%, respectively… However, in all plant tissues tested, both auxins were found to be metabolized rapidly and conjugated at the same rate with amino acids or sugar” (Epstein & Ludwig-Muller, 1993). IBA was also more stable than IAA in several other laboratory experimental conditions. In solutions for adventitious root formation in pea cuttings (Nordstrom et al., 1991), no IAA was detected after 48 hours, but 70% IBA was still found remaining.

Microcuttings may be dipped into rooting powder and planted in soil, or may be rooted *in vitro*. IBA is relatively more stable than IAA (De Klerk et al., 1999). Therefore, IBA was preferred in the former, since the dipping was a one-time application and the effect of IBA as auxin remained relatively longer than IAA. IAA was preferred to be used in the latter *in vitro* rooting, since IBA, being stable and remaining in the system longer than IAA, might prohibit rooting in the third phase and after (De Klerk, 2002).

IBA

Pederson (2007) briefly described the history and function of IBA. IBA was found in 1935 to induce increased rooting in lemon and Chrysanthemum cuttings but was considered to be only “synthetic” at that time (Cooper, 1935). IBA was actually extracted from potato tuber peels in 1954 with the aid of sensitive chemical assays to purify and identify auxins. “Although IBA has been identified as a natural product in many plant species, in many textbooks it is still referred to as a ‘synthetic auxin’,” (Ludwig-Muller, 2000).

“The role of IBA in plant growth regulation is unknown, although it is implicated in root formation and widely used commercially for induction of adventitious rooting,” Normanly et al. (1995). The effect of IBA as a plant hormone is not consistent and its role in plant development is still under debate (Normanly et al., 1995; Ludwig-Muller, 2000; Ludwig-Muller et al., 2005; Ludwig-Muller, 2007; Pederson, 2007; Tognetti et al., 2010; Simon and Petrasek, 2011; Ludwig-Muller, 2011). Nevertheless, using IBA to promote plant growth and plant rooting is a popular application.

Foliar spray of IBA is used in enhancing rooting and crop yield. The application of IBA significantly changed vegetative growth characteristics and increased the yield and yield components of cotton (Sawan et al., 1980), maize (Amin et al., 2006), and other plants (Samananda et al., 1972; Kroin, 1992; Blythe et al., 2004; Khandagale et al., 2009; Abdel, 2011).

IBA is primarily used for rooting many plant species (Ludwig-Muller, 2000). After the effect of auxin, specifically IAA and IBA, was discovered in the 1930s, no new breakthroughs were made in the following 60 years (De Klerk, 2002). The primary operation is by dipping (stem) cuttings in a mixture of talc (a carrier for auxin) and IBA (auxin), or in an IBA solution. IBA was used for stimulating the rooting of hard wood stem cuttings of kiwifruit cv. Hayward (Ercisli et al., 2003), teek (*Tectona grandis* L.f) (Nor Aini et al., 2009), peach (Tworkoski and Takeda, 2007), olive (Sebastiani and Tognetti , 2004; Centeno and Gomez-del-Campo, 2008), mulberry (Koyuncu and Senel, 2003), and apple (Karakurt et al., 2009; Delargy and Wright, 1979). IBA was used for stimulating the lateral roots growth of *Arabidopsis* (Ludwig-Muller et al., 2005; Jemaa et al., 2011), cherry (Christov and Koleva, 1995), and leaf cuttings (Ofori et al., 1996).

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

As being contrasted to the overall pictures of plant propagation and plant hormones given above, IBA is one of numerous plant hormones. Propagation from cuttings is one of numerous plant propagation processes.

Salicylic acid played similar roles as IBA in improving plant growth when salicylic acid and IBA were applied to onion plants (Amin et al., 2007). Diiodosalicylic acid, a compound similar to salicylic acid, is an approved chemical in OMRI as "allowed with restrictions – Synthetic /nonsynthetic" for livestock feed ingredients/livestock health care. Otherwise, no OMRI status is available for using salicylic acid as a plant hormone for organic crop production.

Using juvenile cuttings, supplying sufficient ventilation to remove inhibiting ethylene gas produced during the process, and improving proper water retaining capacity would assist rooting (De Klerk, 2002).

Sugars were found to induce adventitious roots in *Arabidopsis* seedlings (Takahashi et al., 2003) and in *Pelargonium* cuttings (Druege et al., 2004). Sucrose, glucose and fructose greatly stimulated the induction of adventitious roots but mannose and sorbitol did not. Sucrose induced adventitious roots at concentrations of 0.5-2.0%, but suppressed the induction at a concentration of 5%.

The rooting effect of IBA on papaya was enhanced with the presence of 1 μM of riboflavin but roots emerged slowly with riboflavin concentrations greater and less than 1 μM (Drew et al., 1991). The stability of IBA was affected by the presence of riboflavin. The authors (Drew et al., 1991) suggested that this kind of strict requirement might explain variations in results between laboratories and "reduction in auxin concentrations may occur during media preparation and storage."

Successful rooting from stem cuttings depends on numerous factors such as stock plant management, timing, types of cuttings, rooting environment (light, temperature, moisture, etc), and ten other or so factors (Bir and Bilderback, 2011; Hamilton and Midcap, 2009; Ofori et al., 1996). Applying plant hormones is one of these factors. In addition to IBA, NAA is commonly used in conventional operations; however NAA is prohibited under the National Organic Program standards. The application of plant hormones is not a method for all scenarios since this application is further limited by numerous factors: amount, timing, type-mismatch, solution or solid, etc.

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