Hortus USA Corp.

245 WEST 24TH STREET
NEW YORK NY 10011 USA
(212) 929-0927
FAX (212) 624-0202
support@hortus.com • rooting-hormones.com

Primary Supplier of Plant Rooting Products

August 18, 2008

Program Manager
USDA/AMS/TM/NOP
Room 4008-So, Ag Stop 0268
1400 Independence Ave., SW
Washington DC 20250
Phone 202-720-3252
Fax 202-720-7808

Dear Program Manager

Please find attached our Petition of substance for inclusion on the National List of Substances Allowed on Organic Production and Handling.

> Subject active ingredient substance: Indole-3-butyric acid, CAS number 133-32-4.
> Requested as national list substance: As Plant Growth Regulators. Indole-3-butyric acid (IBA).

If you have any questions please contact me directly.

Regards

Joel Kroin
President

TO:

Officer:	Program Manager
Agency:	USDA/AMS/TM/NOP
Address:	Room 4008-So, Ag Stop 0268 1400 Independence Ave., SW Washington DC 20250
Phone:	202-720-3252
Fax:	202-720-7808

FROM PETITIONER:

Contact person:	Joel Kroin
Title:	President
Company:	Hortus USA Corp.
Address:	245 West 24th Street New York NY 10011
Email address:	support@hortus.com
Phone:	212-929-0927
Fax:	212-624-0202

DATE:

Submission:	August 19, 2008

PETITION:

Petition of substances fo inclusion on the National List of Substances allowed on Organic Production and Handling
(7 CFR Part 205, Guidelines on procedures for submitting National List Petitions: AMS-TM-06-0223; TM-06-12)

SUBJECT ACTIVE INGREDIENT SUBSTANCE:

Indole-3-butyric acid	CAS number 133-32-4 Empirical formula C12 H13 NO2

REQUESTED AS NATIONAL LIST SUBSTANCE:

As plant growth regulators. Indole-3-butyric acid (IBA)

INTRODUCTION

Reference:
US EPA Indole-3-butyric acid (046701) Fact Sheet issued August 1, 2000 (attached)

	Indole-3-butyric acid enhances the growth and development of food crops and ornamentals when applied to soil, cuttings, or leaves. Because it is similar in structure to naturally occurring substances and is used in tiny amounts, this plant growth regulator poses no known risks to humans or the environment.
From the US EPA IBA Fact sheet: Description of the Active Ingredient	Indole-3-butyric acid is a substance that is closely related in structure and function to a natural growth regulator found in plants. Indole-3-butyric acid is used on many crops and ornamentals to promote growth and development of roots, flowers and fruits, and to increase crop yields. Growers find it more effective and efficient than its natural counterpart because plants cannot break it down as quickly. No harm to humans or the environment is expected to result from use of Indole-3-butyric acid.
From the US EPA IBA Fact sheet: Use Sites, Target Pests, and Application Methods	Use Sites: Many food and feed crops; ornamental turf and nursery plants Uses: Growth enhancer to increase both yield and quality. Application Methods: Applied to soil or plants as spray. Also used as a dip for cuttings.

From the US EPA IBA Fact sheet: Assessing Risks to Human Health

With the exception of certain workers, no harm is expected from use of indole-3-butyric acid. The active ingredient is not toxic to humans or other mammals.
Furthermore, indole-3-butyric acid is effective at very low concentrations--often several orders of magnitude below 1%.
It is applied at very low rates compared with most other pesticides. In animals, indole-3-butyric acid is rapidly broken down to a closely related, harmless chemical that occurs naturally in living organisms.

Eye irritation to certain workers is EPA' s only health concern for products containing indole-3-butyric acid.
For products that may cause eye irritation, workers (such as mixers and applicators) are required to use protective eye wear, such as goggles, face shield, or safety glasses.

From the US EPA IBA Fact sheet: Assessing Risks to the Environment

No risks to the environment are expected from use of this active ingredient because
1) it does not harm animals or plants in the tiny amounts used,
2) it acts as a plant growth enhancer,
3) it does not persist in the environment,
4) it is closely related to naturally occurring substances.

From the US EPA IBA Fact sheet: Regulatory Information

Products containing indole-3-butyric acid were initially registered (licensed for sale and distribution) in 1960 for use on ornamental plant cuttings and transplants.
As part of EPA' s ongoing review to ensure that pesticide products meet current standards, the chemical was reviewed and found eligible for re-registration in 1992.
As of May 2000, there were more than 40 products containing indole-3-butyric acid as an active ingredient.

From the US EPA IBA Fact sheet: Registrant Information	Many companies have registered pesticide products that contain indole-3-butyric acid. Indole-3-butyric acid enhances the growth and development of food crops and ornamentals when applied to soil, cuttings, or leaves. Because it is similar in structure to naturally occurring substances and is used in tiny amounts, this plant growth regulator poses no known risks to humans or the environment.

PROPOSED CONCLUSIONS FOR PETITION REVIEW BY THE NOSB

(1) potential of such substance for detrimental chemical interactions with other materials used in organic farming systems	None.
(2) toxicity and mode of action of the substance and of its breakdown products or other contaminants and their persistence and areas of concentration in the environment	Low toxicity for both the active ingredient and possible breakdown products. No concern for persistence and areas of concentration in the environment.
(3) probability of environmental contamination during manufacture, use, misuse or disposal of such substances	None when disposed using approved label instructions.
(4) effect of the substance on human health.	None when applied using approved personal protection equipment (PPE).
(5) effects of the substance on biological and chemical interactions in the agroecosystem ...	None. The US EPA has stipulated: "it does not persist in the environment".

(6) alternatives to using the substance in terms of practices or other materials available	No alternative substances are available as US EPA registered plant growth regulator substances. All plant growth regulators are required to be registered with the US EPA under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). There are no state of the art procedures using alternative substances which do not require US EPA registration.
Other verifications:	
Verification of availability of the substance	As of August 16, 2008 US EPA has this substance registered in various formulations by at least 24 companies.
(A) why the substance should be permitted in the production or handling of an organic product.	The substance has a long history to use useful by plant growers. The US EPA has stipulated: "Indole-3-butyric acid is used on many crops and ornamentals to promote growth and development of roots, flowers and fruits, and to increase crop yields. Growers find it more effective and efficient than its natural counterpart because plants cannot break it down as quickly. No harm to humans or the environment is expected to result from use of Indole-3-butyric acid."
(B) The current industry information regarding availability of and history of an organic form in the appropriate form, quality, or quantity of the substance.	(1) There are no organic forms of the substance. the US EPA has ruled that Indole-3-butyric acid, "IBA has been classified as a biochemical pesticide because it is similar in structure and function to the naturally occurring plant growth Indole-3-acetic acid" IAA. There are no US EPA registered substances which contain IAA either as a technical grade product or an end use product. Due to the mode of activity of IAA it is unlikely that manufacturers will register IAA in the future. (2) The substance is manufactured internationally; there is no possibility of loss of availability pf the substance. There are many manufacturers of the substance in the US.

(3) There are always adequate supplies of the substance in commercial trade channels.

(4) There are no trade related issues involving availability of the substance.

(5) There is a ready supply of the substance available to growers at competitive prices.

PETITION

Item A: section the petitioned substance will be included

Synthetic substances allowed for use in organic crop production, 205.601

Item B: information on the substance being petitioned

(1) substance chemical name	**Indole-3-butyric acid**
substance common name	**IBA**
(2) Manufacturer's name:	
Contact person:	Joel Kroin
Title:	President
Company:	Hortus USA Corp.
Address:	245 West 24th Street New York NY 10011
Email address:	support@hortus.com
Phone:	212-929-0927
Fax:	212-624-0202
(3) Intended use	Plant growth regulator
(4) List of crops	Any plants which are propagated from cuttings. Any plants which can benefit from growth enhancement to increase both yield and quality. Many food and feed crops; ornamental turf and nursery plants.
(5) Source of the substance	Substance is a synthesized bio-simulator. Technical grade substance is available from many commercial sources. End use products are compounded from the technical grade substance.
(6) Previous reviews	None are known.
(7) US EPA registration numbers	Rhizopon AA #1, US EPA reg# 63310-19 Rhizopon AA #2 US EPA reg# 63310-20 Rhizopon AA #3 US EPA reg# 63310-21 Rhizopon AA Water Soluble Tablets US EPA reg# 63310-8 Hortus IBA Water Soluble Salts US EPA reg# 63310-22

(8) CAS number product labels

CAS number: 133-32-4

Product labels attached:
US EPA approved stamped product labels for:
Rhizopon AA #1,
US EPA reg# 63310-19
Rhizopon AA #2
US EPA reg# 63310-20
Rhizopon AA #3
US EPA reg# 63310-21
Rhizopon AA Water Soluble Tablets
US EPA reg# 63310-8
Hortus IBA Water Soluble Salts
US EPA reg# 63310-22

(9) substances physical properties

Rhizopon AA #1, Rhizopon AA #2, Rhizopon AA #3 and Hortus IBA Water Soluble Salts are dry powders
Rhizopon AA Water Soluble Tablets is a compounded tablet

Rhizopon AA #1, Rhizopon AA #2, Rhizopon AA #3 are insoluble in water
Hortus IBA Water Soluble Salts are Rhizopon AA Water Soluble Tablets are soluble in water

MSDS with physical properties are attached:
Rhizopon AA #1,
US EPA reg# 63310-19
Rhizopon AA #2
US EPA reg# 63310-20
Rhizopon AA #3
US EPA reg# 63310-21
Rhizopon AA Water Soluble Tablets
US EPA reg# 63310-8
Hortus IBA Water Soluble Salts
US EPA reg# 63310-22

(a) interactions with other substances used in organic production	No interactions. Rhizopon AA Water Soluble Tablets US EPA reg# 63310-8 and Hortus IBA Water Soluble Salts US EPA reg# 63310-22 can be mixed with aqueous solutions of any other substances used in organic production. Rhizopon AA #1, US EPA reg# 63310-19 Rhizopon AA #2, US EPA reg# 63310-20 Rhizopon AA #3, US EPA reg# 63310-21 are insoluble in water and will not mix with other substances used in organic production.

(b) toxicity

Toxicity is listed on the US EPA R.E.D. for IBA (attached).

Toxicity
All generic toxicology data requirements have been waived for IBA. The registered uses result in very low exposure to workers and negligible residues on crops. Products are formulated with very low percentages of IBA (0.0004 to 4.5%) and are applied at ultra-low rates (7 mg IBA/acre). Formulated products generally are of low toxicity. Additionally, IBA is metabolized to 3-indole acetic acid which is a common metabolite in tryptophan (an amino acid) metabolism in humans.

Occupational Exposure
People may be exposed to IBA during mixing, loading and application activities. However, IBA is of low toxicity and is applied at extremely low rates, so exposure data have not been required.
EPA has no significant exposure concerns other than protecting the eyes of mixers, loaders and applicators. Thus, IBA products in Toxicity Category II for primary eye irritation must bear appropriate label precautions, including a requirement that applicators wear protective eyewear (goggles, face shield, or safety glasses).

Human Risk Assessment
The Agency believes the potential risks to humans from occupational and dietary exposure to IBA are negligible.

(c) environmental impact

Environmental impact is listed on the US EPA R.E.D. for IBA (attached)

"Ecological Effects and Environmental Fate
Due to IBA's ultra-low application rates, its behavior as a plant growth hormone, and its similarity in structure and function to other naturally occurring chemicals, EPA believes that IBA poses a negligible risk to the environment."
"Environmental Risk Assessment
EPA concludes for the reasons stated above that the current uses of IBA pose a negligible risk to the environment."

(d) effects on human health

Effects on human health is listed on the US EPA R.E.D. for IBA (attached)

Toxicity

All generic toxicology data requirements have been waived for IBA. The registered uses result in very low exposure to workers and negligible residues on crops. Products are formulated with very low percentages of IBA (0.0004 to 4.5%) and are applied at ultra-low rates (7 mg IBA/acre). Formulated products generally are of low toxicity. Additionally, IBA is metabolized to 3-indole acetic acid which is a common metabolite in tryptophan (an amino acid) metabolism in humans.

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Human Risk Assessment

The Agency believes the potential risks to humans from occupational and dietary exposure to IBA are negligible.

(e) effects on soil organisms, crops or livestock

Effects on plants is listed on the US EPA R.E.D. for IBA (attached)

Use profile:
IBA is a plant growth regulator, used to promote and accelerate root formation of plant clippings and to reduce transplant shock of non-food ornamental nursery stock. IBA is also used on fruit and vegetable crops, field crops and ornamental turf to promote growth development of flowers and fruit and to increase crop yields. IBA has been classified as a biochemical pesticide because it is similar in structure and function to the naturally-occurring plant growth hormone indole-3-acetic acid.

(10) Safety information

(1) MSDS with physical properties and safety information are attached:
Rhizopon AA #1,
US EPA reg# 63310-19
Rhizopon AA #2
US EPA reg# 63310-20
Rhizopon AA #3
US EPA reg# 63310-21
Rhizopon AA Water Soluble Tablets
US EPA reg# 63310-8
Hortus IBA Water Soluble Salts
US EPA reg# 63310-22

(2) Copies of the US EPA stamped labels for US EPA Registered products are attached.
Rhizopon AA #1,
US EPA reg# 63310-19
Rhizopon AA #2
US EPA reg# 63310-20
Rhizopon AA #3
US EPA reg# 63310-21
Rhizopon AA Water Soluble Tablets
US EPA reg# 63310-8
Hortus IBA Water Soluble Salts
US EPA reg# 63310-22

These labels were written for safety information by the US EPA in compliance with the US Department of Labor Worker Protection Safety Act. WPS

(3) Search of the the National Toxicology Program web site
http://ntp-apps.niehs.nih.gov/ntp_tox/index.cfm for the CAS 133-32-4:

"Search found
Found 0 Search Result for Search Term '133-32-4'
No chemical(s) were found based on this search term."

(11) Research information

Attached are selected chapters from:
Peter J. Davies, editor, Plant Hormones, Biosynthesis, Signal Translocation, Action! Kluwer Academic Publishers, Dordrecht, the Netherlands. 2004
(1) The plant hormones: their nature, occurrence and function. Peter J. Davies
(2) Auxin biosynthesis and metabolism. J. Normaly, J. Slovin, J. Cohen

Tim D. Davis, Bruce E. Haisig and Narendra Sankhla. editors. Adventitious root formation in cuttings. Advances in Plant Sciences Series. Volume 2. Dioscorides Press. Portland OR. 1988
(1) Chapter 9. Auxin metabolism during adventitious rooting. Thomas Gaspar and Michel Hofinger
(2) Chapter 10. Chemicals and formulations used to promote adventitious rooting. Frank A. Blazich

(12) Petition justification statement

(a) why the synthetic substance is necessary for the production or handling of an organic product.

Plants can be reproduced in various ways including from seed, from grafts, and from cuttings. Seeds often do not reproduce the next generation as the parent plant. In order to maintain genetic integrity clonal propagation produces new plants that are identical to the parent plant. Plant propagation from cuttings assures that the new generation of plants is identical to the parent plant. While plants generate natural root producing substances, auxins, these substances are not adequate for consistent rooting. Also, many plant varieties are not possible to reproduce from cuttings unless root inducing substances are applied. Indole-3-butyric acid is the most useful rooting inducing substance.

Indole-3-butyric acid is also useful to enhance the root formation in plants that are starting to root but need additional applied root inducing substances to form uniform root systems.

(b) substances on the National List or other cultural methods that could be used in place of the petitioned synthetic substance.

No other substances on the National List are suitable as root inducing substances.

Historically, plant growers for centuries had tried to find root inducing substances that are useful when propagating new plants and to improve plant growth. In the 1930's the natural root inducing substance, IAA, was discovered. When the chemical composition of IAA was identified then other root inducing substances were developed based upon the structure of IAA. Indole-3-butyric acid has been the most successful substance used as a root inducing substance since it is very stable and it is controlled and regulated by the plant. It is useful for root inducing on most plant varieties which can be propagated from cuttings.

(c) beneficial effects to the environment, human health , or farm ecosystem from use of the synthetic substance that support its use instead of the use of a non-synthetic substance or alternative cultural method.

(1) Indole-3-butyric acid has been found to have no hazardous effects to the environment.

The US EPA R.E.D. for IBA (attached).
The US EPA states:
"Ecological Effects and Environmental Fate
Due to IBA's ultra-low application rates, its behavior as a plant growth hormone, and its similarity in structure and function to other naturally occurring chemicals, EPA believes that IBA poses a negligible risk to the environment."

(2) Indole-3-butyric acid has been shown to be non-hazardous to humans when used in accordance to standard personal protection procedures.

The US EPA R.E.D. for IBA (attached).
The US EPA states:
"Human Risk Assessment.
The Agency believes the potential risks to humans from occupational and dietary exposure to IBA are negligible."

(3) There are no other non-synthetic substances or alternative cultural methods available.

ATTACHMENTS

Peter J. Davies, editor. Plant Hormones, Biosynthesis, Signal Translocation, Action!
Kluwer Academic Publishers, Dordrecht, the Netherlands. 2004
(1) The plant hormones: their nature, occurrence and function. Peter J. Davies
(2) Auxin biosynthesis and metabolism. J. Normaly, J. Slovin, J. Cohen

Tim D. Davis, Bruce E. Haisig and Narendra Sankhla. editors.
Adventitious root formation in cuttings.
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(1) Chapter 9. Auxin metabolism during adventitious rooting. Thomas Gaspar and Michel Hofinger
(2) Chapter 10. Chemicals and formulations used to promote adventitious rooting. Frank A. Blazich

MSDS with physical properties and safety:
- Rhizopon AA #1, US EPA reg# 63310-19
- Rhizopon AA #2, US EPA reg# 63310-20
- Rhizopon AA #3, US EPA reg# 63310-21
- Rhizopon AA Water Soluble Tablets, US EPA reg# 63310-8
- Hortus IBA Water Soluble Salts, US EPA reg# 63310-22

US EPA stamped approval labels:
- Rhizopon AA #1, US EPA reg# 63310-19
- Rhizopon AA #2, US EPA reg# 63310-20
- Rhizopon AA #3, US EPA reg# 63310-21
- Rhizopon AA Water Soluble Tablets, US EPA reg# 63310-8
- Hortus IBA Water Soluble Salts, US EPA reg# 63310-22

- US EPA R.E.D Facts: Indole-3-butyric acid. August 1992

- US EPA R.E.D Eligibility Requirements: Indole-3-butyric acid. August 1992

- US EPA FIFRA: definitions and registration requirements

- US EPA registered IBA containing products by product name: 2008

- US EPA registrants of IBA containing products: 2008

- US EPA registered active ingredients, alphabetical list 2008

RHIZOPON®

AA #1 (0.1)

PINK POWDER COLOR CODED
FOR EASY IDENTIFICATION
Plant Growth Regulator-
Dry Powder Rooting Hormone

USE TO PROMOTE ROOTING
OF PLANT CUTTINGS
For Rooting House, Foliage, Tropical
and Hardy Ornamental Plants;
Leaf, Greenwood and Softwood Cuttings

Use in Greenhouses and Nurseries Only

Ingredients:	
Active ingredients	
Indole-3-butyric acid	0.1%
Other Ingredients	99.9%
Total	100.0%

Exclusive USA Importer: Hortus USA Corp., NY NY 10011
Made in Holland. EPA Reg No. 63310-19. EPA Est No. 63310-HL-001
Master USA Distributor: PHYTOTRONICS, 314-770-0717

Net Wt: 1 lb.

Keep Out Of Reach Of Children

CAUTION

See additional precautionary statements on back panels

Use Rhizopon AA to propagate new plants from cuttings. The new plants will have the same characteristics as the parent plant. Rhizopon AA treated cuttings will expect to produce uniform roots all around the basal end of the cuttings; the results are beautiful symmetric plants.

Rhizopon AA #1 is used on most home, tropical, green house and nursery plants including herbaceous and soft wood cuttings.

Typical plants propagated using Rhizopon #1 (0.1):

African violet, ageratum, andromeda, azalea var., barberry, bayberry, beauty berry, begonia, blackberry, bluebeard, blueberry, bougainvillea, snake plant, broom, abelia, butterfly bush, candytuft, carnation, chrysanthemum, clerodendron, clockvine, coleus, crape myrtle, crassula, creeper, croton, currant, daphne, deutzia, dewberry, dovetree, dracena, dutchmanspipe, elder, firethorne, flowering cherry var., fontanesia, forsythia, fuchsia, gardenia, geranium, hazelnut, jasmine, jetbead, kerria, laburnocytisus, lantana, lily scales, linden, melastoma, mock orange, mulberry, orixa, osage orange, pea shrub, penstemon, philodendron, phlox, poinsettia, poplar, pricklypear cactus, raspberry, rose, sage, snapdragon, snowberry, speedwell, spiraea, Norway spruce var., stevia, stewartia, St. Johnswort, sweetleaf, trumpet creeper, verbena, viburnum, waxmyrtle, weigelia, willow.

ACCEPTED

OCT 10 2006

Under the Federal Insecticide, Fungicide, and Rodenticide Act, as amended, for the pesticide registered under EPA Reg. No. 63310-19

PRECAUTIONARY STATEMENTS
Hazards to humans and domestic animals.

CAUTION
Causes moderate eye injury. Harmful if inhaled or absorbed through the skin. Avoid contact with eyes, skin, ingestion or inhalation. Avoid breathing dust (vapor or spray mist). Wash thoroughly with soap and water after handling. Remove contaminated clothing and wash before reuse.

PERSONAL PROTECTIVE EQUIPMENT
Applicators and other handlers must wear: long sleeve shirt, long pants, shoes, socks and waterproof gloves, and for exposure in enclosed areas or outdoors, dust/mist filtering respirator. (MSHA/NIOSH approval number prefix TC-21C) or a NIOSH-approved respirator with any R, P or HE filter.

USER SAFETY RECOMMENDATIONS
Users should: Wash hands before eating, drinking, chewing gum, using tobacco or using the toilet. Remove clothing/PPE immediately if pesticide gets inside. Then wash thoroughly and put on clean clothing.
Remove PPE immediately after handling this product. Wash the outside of gloves before removing. As soon as possible, wash thoroughly and change into clean clothing.

FIRST AID Classification of Pesticide: Plant Growth Regulator	
If in eyes	> Hold eyes open and rinse slowly with water for 15-20 minutes > Remove contact lenses, if present after the first 5 minutes then continue rinsing eye > Call a poison center or a doctor for further treatment or advise
If on skin or clothing	> Take off contaminated clothing > Rinse skin immediately with plenty of water for 15-20 minutes > Call a poison center or a doctor for further treatment or advise
If swallowed	> Call a poison center or a doctor for further treatment or advise > Have person sip water if able to swallow > Do not induce vomiting unless told to do so by the poison control center or doctor. > Do not give anything by mouth to an unconscious person.
If inhaled	> Move person to fresh air > If person is not breathing call 911 or an ambulance, then give artificial respiration, preferably by mouth to mouth, if possible > Call a poison center or a doctor for further treatment or advise
NOTE TO PHYSICIAN: May cause minor eye irritation which will last a short time. This product does NOT contain any petroleum, caustics or active solvent products	
Have the product container or label with you when you call a poison control center or doctor or going for treatment. You may call 800-325-3055 or 314-770-0717. 7:30AM-5PM CST Mon-Fri or the National Pesticide Information Center (NPIC) at 1-800-858-7378	

You may also call 800-325-3055 or 314-770-0717 for an MSDS. 7:30AM-5PM CST M-F

ENVIRONMENTAL HAZARDS
For terrestrial uses. Do not apply directly to water, or to areas where surface water is present or to intertidal areas below the mean high water mark. Do not contaminate water by cleaning of equipment or disposal of equipment wash waters or rinsate.

DIRECTIONS FOR USE

It is a violation of Federal law to use this product in a manner inconsistent with labeling. For any requirements specific to your State or Tribe, consult the agency responsible.

AGRICULTURAL USE REQUIREMENTS
Use this product in accordance with its labeling and with the Worker Protection Standard, 40 CFR 170. This Standard contains requirements for the protection of agricultural workers on farms, forests nurseries, and greenhouses, and handlers of agricultural pesticides. It contains requirements for training, decontamination, notification, and emergency assistance. It also contains specific instructions and exceptions pertaining to the statements on this labeling about personal protective equipment and restricted-entry intervals. The requirements in this box only apply to the uses of this product that are covered by the Workers Protection Standard (WPS).
Do not apply this product in a way that will contact workers or other persons, either directly or through drift. Only protected handlers may be in the area during application.
THE RESTRICTED-ENTRY INTERVAL (REI)
FOR THIS PRODUCT IS "0" HOURS.

1. Take plant cuttings, usually 4-6 inch stem cuttings, from the current years growth. For woody cuttings usually wound by making a 3/4 inch notch at basal end.
2. Dip the basal end of the cuttings 3/4-1 inch into the Rhizopon AA powder. Tap off excess powder.
3. Plant the cuttings immediately in moist media. Avoid contact between the powder and foliage and other over ground parts of the stem.
4. Take care of cuttings as appropriate for the plant variety. Control watering, temperature, humidity, light and other environmental factors. Observe and control external factors such as insects and fungus.

Take off a small portion of the powder for your immediate use. Do not contaminate this stock container by returning used portion to this container. Dispose of un-used portion as described in the 'Storage and Disposal' statements on this label.

STORAGE AND DISPOSAL
Do not contaminate water, food, or feed by storage and disposal.
Pesticide Storage: Store in a cool dry place. Keep in original container.
Pesticide Disposal: Wastes resulting from this product may be disposed of on site or at an approved waste disposal facility.
Container Disposal: Triple rinse (or equivalent). Then offer for recycling, or reconditioning, or puncture and dispose of in a sanitary landfill, or incineration, or, if allowed by state and local authorities, by burning. If burned stay out of smoke.

WARRANTY
It is warranted that this product conforms to the chemical description on the label thereof and is reasonably fit for purposes stated on such label only when used in accordance with the directions under normal use conditions. It is impossible to eliminate all risks inherently associated with the use of this product. Crop injury, ineffectiveness or other unintended consequences may result because of such factors as weather conditions, presence of other materials, or the manner of application all of which is beyond the control of Rhizopon b.v. or Hortus USA Corp. To the extent of the law, Rhizopon or Hortus USA shall not be liable for consequential, special or indirect damages resulting from the use or handling of this product. To the extent of the law, all risks shall be assumed by the buyer. Rhizopon or Hortus USA make no warranties of merchantability or fitness for a particular purpose nor other express or implied warranty except as stated above.

Hortus USA Corp., PO Box 1956, New York, NY 10113
Master Distributor: Phytotronics
13688 Rider Trail N, Earth City MO 63045 USA.
Phone: 314-770-0717
www.rooting-hormones.com

PRECAUTIONARY STATEMENTS
Hazards to humans and domestic animals.

CAUTION
Causes moderate eye injury. Harmful if inhaled or absorbed through the skin. Avoid contact with eyes, skin, ingestion or inhalation. Avoid breathing dust (vapor or spray mist). Wash thoroughly with soap and water after handling. Remove contaminated clothing and wash before reuse.

PERSONAL PROTECTIVE EQUIPMENT
Applicators and other handlers must wear: long sleeve shirt, long pants, shoes, socks and waterproof gloves, and for exposure in enclosed areas or outdoors, dust/mist filtering respirator. (MSHA/NIOSH approval number prefix TC-21C) or a NIOSH-approved respirator with any R, P or HE filter.

USER SAFETY RECOMMENDATIONS
Users should: Wash hands before eating, drinking, chewing gum, using tobacco or using the toilet. Remove clothing/PPE immediately if pesticide gets inside. Then wash thoroughly and put on clean clothing.
Remove PPE immediately after handling this product. Wash the outside of gloves before removing. As soon as possible, wash thoroughly and change into clean clothing.

FIRST AID Classification of Pesticide: Plant Growth Regulator	
If in eyes	> Hold eyes open and rinse slowly with water for 15-20 minutes > Remove contact lenses, if present after the first 5 minutes then continue rinsing eye > Call a poison center or a doctor for further treatment or advise
If on skin or clothing	> Take off contaminated clothing > Rinse skin immediately with plenty of water for 15-20 minutes > Call a poison center or a doctor for further treatment or advise
If swallowed	> Call a poison center or a doctor for further treatment or advise > Have person sip water if able to swallow > Do not induce vomiting unless told to do so by the poison control center or doctor. > Do not give anything by mouth to an unconscious person.
If inhaled	> Move person to fresh air > If person is not breathing call 911 or an ambulance, then give artificial respiration, preferably by mouth to mouth, if possible > Call a poison center or a doctor for further treatment or advise
NOTE TO PHYSICIAN: May cause minor eye irritation which will last a short time. This product does NOT contain any petroleum, caustics or active solvent products	
Have the product container or label with you when you call a poison control center or doctor or going for treatment. You may call 800-325-3055 or 314-770-0717, 7:30AM-5PM CST Mon-Fri or the National Pesticide Information Center (NPIC) at 1-800-858-7378	

You may also call 800-325-3055 or 314-770-0717 for an MSDS. 7:30AM-5PM CST M-F

ENVIRONMENTAL HAZARDS
For terrestrial uses. Do not apply directly to water, or to areas where surface water is present or to intertidal areas below the mean high water mark. Do not contaminate water by cleaning of equipment or disposal of equipment wash waters or rinsate.

DIRECTIONS FOR USE

It is a violation of Federal law to use this product in a manner inconsistent with labeling. For any requirements specific to your State or Tribe, consult the agency responsible.

AGRICULTURAL USE REQUIREMENTS
Use this product in accordance with its labeling and with the Worker Protection Standard, 40 CFR 170. This Standard contains requirements for the protection of agricultural workers on farms, forests nurseries, and greenhouses, and handlers of agricultural pesticides. It contains requirements for training, decontamination, notification, and emergency assistance. It also contains specific instructions and exceptions pertaining to the statements on this labeling about personal protective equipment and restricted-entry intervals. The requirements in this box only apply to the uses of this product that are covered by the Workers Protection Standard (WPS).
Do not apply this product in a way that will contact workers or other persons, either directly or through drift. Only protected handlers may be in the area during application.
THE RESTRICTED-ENTRY INTERVAL (REI)
FOR THIS PRODUCT IS "0" HOURS.

1. Take plant cuttings, usually 4-6 inch stem cuttings, from the current years growth. For woody cuttings usually wound by making a 3/4 inch notch at basal end.
2. Dip the basal end of the cuttings 3/4-1 inch into the Rhizopon AA powder. Tap off excess powder.
3. Plant the cuttings immediately in moist media. Avoid contact between the powder and foliage and other over ground parts of the stem.
4. Take care of cuttings as appropriate for the plant variety. Control watering, temperature, humidity, light and other environmental factors. Observe and control external factors such as insects and fungus.

Take off a small portion of the powder for your immediate use. Do not contaminate this stock container by returning used portion to this container. Dispose of un-used portion as described in the 'Storage and Disposal' statements on this label.

STORAGE AND DISPOSAL
Do not contaminate water, food, or feed by storage and disposal.
Pesticide Storage: Store in a cool dry place. Keep in original container.
Pesticide Disposal: Wastes resulting from this product may be disposed of on site or at an approved waste disposal facility.
Container Disposal: Triple rinse (or equivalent). Then offer for recycling, or reconditioning, or puncture and dispose of in a sanitary landfill, or incineration, or, if allowed by state and local authorities, by burning. If burned stay out of smoke.

WARRANTY
It is warranted that this product conforms to the chemical description on the label thereof and is reasonably fit for purposes stated on such label only when used in accordance with the directions under normal use conditions. It is impossible to eliminate all risks inherently associated with the use of this product. Crop injury, ineffectiveness or other unintended consequences may result because of such factors as weather conditions, presence of other materials, or the manner of application all of which is beyond the control of Rhizopon b.v. or Hortus USA Corp. To the extent of the law, Rhizopon or Hortus USA shall not be liable for consequential, special or indirect damages resulting from the use or handling of this product. To the extent of the law, all risks shall be assumed by the buyer. Rhizopon or Hortus USA make no warranties of merchantability or fitness for a particular purpose nor other express or implied warranty except as stated above.

Hortus USA Corp., PO Box 1956, New York, NY 10113
Master Distributor: Phytotronics
13688 Rider Trail N, Earth City MO 63045 USA.
Phone: 314-770-0717
www.rooting-hormones.com

RHIZOPON®

AA #2 (0.3)

GREEN POWDER COLOR CODED
FOR EASY IDENTIFICATION

Plant Growth Regulator-
Dry Powder Rooting Hormone

USE TO PROMOTE ROOTING
OF PLANT CUTTINGS
For Rooting Herbaceous, Greenwood,
Softwood and Hardwood Cuttings

Use in Greenhouses and Nurseries Only

Ingredients:	
Active Ingredients	
Indole-3-butyric acid	0.3%
Other ingredients	99.7%
Total	100.0%

Exclusive USA Importer: Hortus USA Corp., NY NY 10011
Made in Holland. EPA Reg No. 63310-20. EPA Est No. 63310-HL-001
Master USA Distributor: PHYTOTRONICS, 314-770-0717

Net Wt: 1 lb.

Keep Out Of Reach Of Children

CAUTION

See additional precautionary statements on back panels

Use Rhizopon AA to propagate new plants from cuttings. The new plants will have the same characteristics as the parent plant. Rhizopon AA treated cuttings will expect to produce uniform roots all around the basal end of the cuttings; the results are beautiful symmetric plants.

Rhizopon AA #2 is used on herbaceous, greenwood, softwood and hardwood cuttings.

Typical plants propagated using Rhizopon #2 (0.3):

apple (malus sp.), arborvitae, ardisia, azalea var., beech, blueberry, broom, chokeberry, cinquefoil, clematis, crabapple, dahlia, daphne, euonymus, elder, false arborvitae, firethorne, franklinia, fringe tree, gardenia, germander, ginkgo, golden chain, hazelnut, hemlock var., hibiscus, holly Jap., honeysuckle, hydrangea, juniper var., laburnocytisus, lavender, leucothoe, lily scales, magnolia, oleander, osmanthus, pachysandra, periwinkle, petunia, pine var., sequoia, silverbell, springscent, spruce var., wintergreen, wisteria, witch hazel, yellow wood, zelkova.

ACCEPTED

OCT 10 2006

Under the Federal Insecticide, Fungicide, and Rodenticide Act, as amended, for the pesticide registered under EPA Reg. No. 63310-20

RHIZOPON®

AA #2 (0.3)

GREEN POWDER COLOR CODED
FOR EASY IDENTIFICATION

Plant Growth Regulator-
Dry Powder Rooting Hormone

USE TO PROMOTE ROOTING
OF PLANT CUTTINGS
For Rooting Herbaceous, Greenwood,
Softwood and Hardwood Cuttings

Use in Greenhouses and Nurseries Only

Ingredients:	Active ingredients
Indole-3-butyric acid	0.3%
Other ingredients	99.7%
Total	100.0%

Exclusive USA Importer: Hortus USA Corp., NY NY 10011
Made in Holland. EPA Reg No. 63310-20. EPA Est No. 63310-HL-001
Master USA Distributor: PHYTOTRONICS, 314-770-0717

Net Wt: 1 lb.

Keep Out Of Reach Of Children

CAUTION

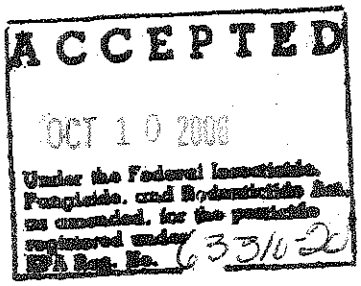
See additional precautionary statements on back panels

Use Rhizopon AA to propagate new plants from cuttings. The new plants will have the same characteristics as the parent plant. Rhizopon AA treated cuttings will expect to produce uniform roots all around the basal end of the cuttings; the results are beautiful symmetric plants.

Rhizopon AA #2 is used on herbaceous, greenwood, softwood and hardwood cuttings.

Typical plants propagated using Rhizopon #2 (0.3):

apple (malus sp.), arborvitae, ardisia, azalea var., beech, blueberry, broom, chokeberry, cinquefoil, clematis, crabapple, dahlia, daphne, euonymus, elder, false arborvitae, firethorne, franklinia, fringe tree, gardenia, germander, ginkgo, golden chain, hazelnut, hemlock var., hibiscus, holly Jap., honeysuckle, hydrangea, juniper var., laburnocytisus, lavender, leucothoe, lily scales, magnolia, oleander, osmanthus, pachysandra, periwinkle, petunia, pine var., sequoia, silverbell, springscent, spruce var., wintergreen, wisteria, witch hazel, yellow wood, zelkova.



RHIZOPON®

AA #3 (0.8)

WHITE POWDER COLOR CODED
FOR EASY IDENTIFICATION
Plant Growth Regulator-
Dry Powder Rooting Hormone

USE TO PROMOTE ROOTING
OF PLANT CUTTINGS
For Rooting House, Foliage, Tropical
and Hardy Ornamental Plants;
Leaf, Greenwood and Softwood Cuttings

Use in Greenhouses and Nurseries Only

Ingredients:	Active ingredients
Indole-3-butyric acid	0.8%
Other ingredients	99.2%
Total	100.0%

Exclusive USA importer: Hortus USA Corp., NY NY 10011
Made in Holland. EPA Reg No. 63310-21. EPA Est No. 63310-HL-001
Master USA Distributor: PHYTOTRONICS, 314-770-0717

Net Wt: 1 lb.

Keep Out Of Reach Of Children

CAUTION

See additional precautionary statements on back panels

Use Rhizopon AA to propagate new plants from cuttings. The new plants will have the same characteristics as the parent plant. Rhizopon AA treated cuttings will expect to produce uniform roots all around the basal end of the cuttings; the results are beautiful symmetric plants.

Rhizopon AA #3 is used on greenwood, softwood and hardwood cuttings including more difficult to root cuttings.

Typical plants propagated using Rhizopon #3 (0.8):

acanthropanax, apple (malus sp.), arborvitae, arbutus, azalea var., beauty bush, birch, bittersweet, boxwood, camellia, catalpa, chaste tree, chestnut, choke berry, cotoneaster, crab apple, cryptomeria, dogwood, Douglas fir, escallonia, flowering quince, gardenia, germander, grape, hawthorn, heath, heather, hemlock var., hibiscus, holly (Am., Chin., Eng.), juniper var., knotwood, laurel, lilac, locust, magnolia, manzanita, maple Jap. var., matrimony vine, ninebark, oak, olive, orange (sour), pear rootstocks, pachysandra, pecan, petunia, photinia, pine var., privet, retinospora, rhododendron var., Russian olive, snowbell, sourwood, tulip tree, umbrella pine, yew var.

ACCEPTED

OCT 1 0 2006

Under the Federal Insecticide, Fungicide, and Rodenticide Act, as amended, for the pesticide registered under EPA Reg. No. 63310-21

PRECAUTIONARY STATEMENTS
Hazards to humans and domestic animals.

CAUTION
Causes moderate eye injury. Harmful if inhaled or absorbed through the skin. Avoid contact with eyes, skin, ingestion or inhalation. Avoid breathing dust (vapor or spray mist). Wash thoroughly with soap and water after handling. Remove contaminated clothing and wash before reuse.

PERSONAL PROTECTIVE EQUIPMENT
Applicators and other handlers must wear: long sleeve shirt, long pants, shoes, socks and waterproof gloves, and for exposure in enclosed areas or outdoors, dust/mist filtering respirator. (MSHA/NIOSH approval number prefix TC-21C) or a NIOSH-approved respirator with any R, P or HE filter.

USER SAFETY RECOMMENDATIONS
Users should: Wash hands before eating, drinking, chewing gum, using tobacco or using the toilet. Remove clothing/PPE immediately if pesticide gets inside. Then wash thoroughly and put on clean clothing.
Remove PPE immediately after handling this product. Wash the outside of gloves before removing. As soon as possible, wash thoroughly and change into clean clothing.

FIRST AID Classification of Pesticide: Plant Growth Regulator	
If in eyes	> Hold eyes open and rinse slowly with water for 15-20 minutes > Remove contact lenses, if present after the first 5 minutes then continue rinsing eye > Call a poison center or a doctor for further treatment or advise
If on skin or clothing	> Take off contaminated clothing > Rinse skin immediately with plenty of water for 15-20 minutes > Call a poison center or a doctor for further treatment or advise
If swallowed	> Call a poison center or a doctor for further treatment or advise > Have person sip water if able to swallow > Do not induce vomiting unless told to do so by the poison control center or doctor. > Do not give anything by mouth to an unconscious person.
If inhaled	> Move person to fresh air > If person is not breathing call 911 or an ambulance, then give artificial respiration, preferably by mouth to mouth, if possible > Call a poison center or a doctor for further treatment or advise
NOTE TO PHYSICIAN: May cause minor eye irritation which will last a short time. This product does NOT contain any petroleum, caustics or active solvent products	
Have the product container or label with you when you call a poison control center or doctor or going for treatment. You may call 800-325-3055 or 314-770-0717. 7:30AM-5PM CST Mon-Fri or the National Pesticide Information Center (NPIC) at 1-800-858-7378	

You may also call 800-325-3055 or 314-770-0717 for an MSDS. 7:30AM-5PM CST M-F

ENVIRONMENTAL HAZARDS
For terrestrial uses. Do not apply directly to water, or to areas where surface water is present or to intertidal areas below the mean high water mark. Do not contaminate water by cleaning of equipment or disposal of equipment wash waters or rinsate.

DIRECTIONS FOR USE

It is a violation of Federal law to use this product in a manner inconsistent with labeling. For any requirements specific to your State or Tribe, consult the agency responsible for pesticide regulation..

AGRICULTURAL USE REQUIREMENTS
Use this product in accordance with its labeling and with the Worker Protection Standard, 40 CFR 170. This Standard contains requirements for the protection of agricultural workers on farms, forests nurseries, and greenhouses, and handlers of agricultural pesticides. It contains requirements for training, decontamination, notification, and emergency assistance. It also contains specific instructions and exceptions pertaining to the statements on this labeling about personal protective equipment and restricted-entry intervals. The requirements in this box only apply to the uses of this product that are covered by the Workers Protection Standard (WPS).
Do not apply this product in a way that will contact workers or other persons, either directly or through drift. Only protected handlers may be in the area during application.
THE RESTRICTED-ENTRY INTERVAL (REI)
FOR THIS PRODUCT IS "0" HOURS.

1. Take plant cuttings, usually 4-6 inch stem cuttings, from the current years growth. For woody cuttings usually wound by making a 3/4 inch notch at basal end.
2. Dip the basal end of the cuttings 3/4-1 inch into the Rhizopon AA powder. Tap off excess powder.
3. Plant the cuttings immediately in moist media. Avoid contact between the powder and foliage and other over ground parts of the stem.
4. Take care of cuttings as appropriate for the plant variety. Control watering, temperature, humidity, light and other environmental factors. Observe and control external factors such as insects and fungus.

Take off a small portion of the powder for your immediate use. Do not contaminate this stock container by returning used portion to this container. Dispose of un-used portion as described in the 'Storage and Disposal' statements on this label.

STORAGE AND DISPOSAL
Do not contaminate water, food, or feed by storage and disposal.
Pesticide Storage: Store in a cool dry place. Keep in original container.
Pesticide Disposal: Wastes resulting from this product may be disposed of on site or at an approved waste disposal facility.
Container Disposal: Triple rinse (or equivalent). Then offer for recycling, or reconditioning, or puncture and dispose of in a sanitary landfill, or incineration, or, if allowed by state and local authorities, by burning. If burned stay out of smoke.

WARRANTY
It is warranted that this product conforms to the chemical description on the label thereof and is reasonably fit for purposes stated on such label only when used in accordance with the directions under normal use conditions. It is impossible to eliminate all risks inherently associated with the use of this product. Crop injury, ineffectiveness or other unintended consequences may result because of such factors as weather conditions, presence of other materials, or the manner of application all of which is beyond the control of Rhizopon b.v. or Hortus USA Corp. To the extent of the law, Rhizopon or Hortus USA shall not be liable for consequential, special or indirect damages resulting from the use or handling of this product. To the extent of the law, all risks shall be assumed by the buyer. Rhizopon or Hortus USA make no warranties of merchantability or fitness for a particular purpose nor other express or implied warranty except as stated above.

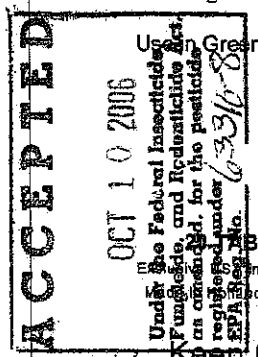
Hortus USA Corp., PO Box 1956, New York, NY 10113
Master Distributor: Phytotronics
13688 Rider Trail N, Earth City MO 63045 USA.
Phone: 314-770-0717
www.rooting-hormones.com

RHIZOPON® AA WATER SOLUBLE TABLETS

Plant Growth Regulator - Rooting Hormone

Propagate New Plants by Rooting Cuttings.
Improve Transplanting - Promote Root Regeneration.
Improve Plant Growth & Flower Yields.
Build Root Mass

Use on house, tropical, herbaceous, perennial & foliage plants, hardy ornamental plants, trees & shrubs, evergreen and deciduous plants.



Use in Greenhouses and Nurseries Only

Active Ingredients:
Indole-3-butyric Acid (50 mg/tablet)....20.0%
Other Ingredients....80.0%
Total...100.0%

Contents:
24 TABLETS. 250 mg each. Net wt. 5 grams

Exclusive U.S. Importer: HORTUS USA Corp., New York NY 10011
Manufactured for: EPA REG # 63310-8 EPA EST # 63310-HL-001

Keep Out Of Reach Of Children
CAUTION

See Additional Precautionary Statements on Back Panel

For Technical Support visit
www.rooting-hormones.com

Use Rhizopon AA to propagate new plants from cuttings. The new plants will have the same characteristics as the parent plant. Rhizopon AA treated cuttings will expect to produce uniform roots all around the basal end of the cuttings; the results are beautiful symmetric plants.

PRECAUTIONARY STATEMENTS
Hazards to humans and domestic animals.
CAUTION
Causes moderate eye injury. Harmful if inhaled or absorbed through the skin. Avoid contact with eyes, skin, ingestion or inhalation. Avoid breathing dust (vapor or spray mist). Wash thoroughly with soap and water after handling. Remove contaminated clothing and wash before reuse.

PERSONAL PROTECTIVE EQUIPMENT
Applicators and other handlers must wear: long sleeve shirt, long pants, shoes, socks and waterproof gloves, and for exposure in enclosed areas or outdoors, dust/mist filtering respirator, (MSHA/NIOSH approval number prefix TC-21C) or a NIOSH-approved respirator with any R, P or HE filter.

USER SAFETY RECOMMENDATIONS
Users should: Wash hands before eating, drinking, chewing gum, using tobacco or using the toilet. Remove clothing/PPE immediately if pesticide gets inside. Then wash thoroughly and put on clean clothing.
Remove PPE immediately after handling this product. Wash the outside of gloves before removing. As soon as possible, wash thoroughly and change into clean clothing.

FIRST AID Classification of Pesticide: Plant Growth Regulator	
If in eyes	> Hold eyes open and rinse slowly with water for 15-20 minutes > Remove contact lenses, if present after the first 5 minutes then continue rinsing eye > Call a poison center or a doctor for further treatment or advise
If on skin or clothing	> Take off contaminated clothing > Rinse skin immediately with plenty of water for 15-20 minutes > Call a poison center or a doctor for further treatment or advise
If swallowed	> Call a poison center or a doctor for further treatment or advise > Have person sip water if able to swallow > Do not induce vomiting unless told to do so by the poison control center or doctor. > Do not give anything by mouth to an unconscious person.
If inhaled	> Move person to fresh air > If person is not breathing call 911 or an ambulance, then give artificial respiration, preferably by mouth to mouth, if possible > Call a poison center or a doctor for further treatment or advise
NOTE TO PHYSICIAN: May cause minor eye irritation which will last a short time. This product does NOT contain any petroleum, caustics or active solvent products	
Have the product container or label with you when you call a poison control center or doctor or going for treatment. You may call 800-325-3055 or 314-770-0717. 7:30AM-5PM CST Mon-Fri or the National Pesticide Information Center (NPIC) at 1-800-858-7378	

You may also call 800-325-3055 or 314-770-0717 for an MSDS. 7:30AM-5PM CST Mon-Fri

ENVIRONMENTAL HAZARDS
For terrestrial uses. Do not apply directly to water, or to areas where surface water is present or to intertidal areas below the mean high water mark. Do not contaminate water by cleaning of equipment or disposal of equipment wash waters or rinsate.

DIRECTIONS FOR USE

It is a violation of Federal law to use this product in a manner inconsistent with labeling. For any requirements specific to your State or Tribe, consult the agency responsible for pesticide regulation.

AGRICULTURAL USE REQUIREMENTS

Use this product in accordance with its labeling and with the Worker Protection Standard, 40 CFR 170. This Standard contains requirements for the protection of agricultural workers on farms, forests nurseries, and greenhouses, and handlers of agricultural pesticides. It contains requirements for training, decontamination, notification, and emergency assistance. It also contains specific instructions and exceptions pertaining to the statements on this labeling about personal protective equipment and restricted-entry intervals.

The requirements in this box only apply to the uses of this product that are covered by the Workers Protection Standard (WPS).

Do not apply this product in a way that will contact workers or other persons, either directly or through drift. Only protected handlers may be in the area during application.

THE RESTRICTED-ENTRY INTERVAL (REI)
FOR THIS PRODUCT IS "0" HOURS.

RATE DETERMINATION

A wide dosage range is indicated for most uses of this product. The ideal rate will vary according to specific plant variety and local growing conditions. To assure compatibility of this product under the specific growing conditions and to determine the dosage rate best suited for your growing conditions, it is recommended that you test a few plants at varying rates within the specified dosage range prior to large scale operations. If combined with other plant regulators, test varying rates of each product. If foliar application causes phytotoxicity, try soil applications or decreased rates.

NOTES
'AI' is the Active Ingredient specified as 'ppm AI
'= parts per million AI
= milligrams (mg) AI per liter (L).
1 Liter (L)= 1000 milliliter (ml)= 33.8 fluid ounces.
For additional information on how to use 'Active Ingredient' see 'To Make a Solution of Any Working Volume'.

PROMOTE ROOTING OF PLANT CUTTINGS

Use to propagate new plants from cutting- from those easy to root to the most difficult to root.

Apply to plant cuttings by convenient methods:

Total Immerse
Completely immerse several cuttings at a time for a few seconds then plant.

Quick Dip
Immerse basal end of cuttings for a few seconds then plant.

Spray Drip Down ™
Plant many cuttings in trays then spray cuttings until drips down.

Immersion Method
Immerse basal end of cuttings for a few hours then plant.

1. Take plant cuttings, usually stem cuttings. For woody cuttings usually wound by making a 3/4" notch at basal end.
2. For Quick Dip and Immerse treat basal end of plant cuttings with solution then plant. For Total Immerse dip whole cuttings in solution then plant. For spray Drip Down plant cuttings then treat by spraying leaves and stems until drip down.
3. Take care of cuttings as appropriate for the plant variety. Control watering, temperature, humidity, light and other environmental factors. Observe and control external factors such as insects and fungus.

Mix a portion of the solution for the same day use. Dispose of un-used portion as described in the 'storage and disposal' statements on this label.

IMMERSION METHOD

Immerse Basal End Then Plant

Immerse basal end of cuttings approximately 1" in solution for 4 to12 hours. Plant immediately.

Herbaceous and Woody Cuttings, including hard to root cuttings and rootstocks: 1-3 Tablets/liter water (50-150 ppm AI)

QUICK DIP METHOD

Immerse Basal End Then Plant

Immerse basal end of cuttings approximately 1" in solution a few seconds. Plant immediately.

To mix more than 10 Tablets/liter see 'To Make a Solution'.

Herbaceous Cuttings, Tropical Plants, House Plants, Roses, etc.: 3-10 Tablets/liter water (150-500 ppm AI)

Chrysanthemum: 4-10 Tablets/liter water (200-500 ppm AI)

Softwood Cuttings: 20 Tablets/liter (1000 ppm AI)

Hardwood Cuttings: 40 Tablets/liter (2000 ppm AI)

Difficult to Root Cuttings: 100-400 Tablets/liter (5000-20000 ppm AI)

TOTAL IMMERSE METHOD

Total Immerse Cuttings Then Plant

Immerse whole cuttings in solution a few seconds. A basket is useful. Plant immediately.

Herbaceous Cuttings, Chrysanthemum, Plumbago, Ivy, Clematis, Delphinium, Lavender, Ficus, Potted Rose Bushes, etc.:1-5 Tablets/liter water (50-250 ppm AI)

Softwood Cuttings, Buddleia (Butterfly Bush), Hydrangea, Pieris, Hedera (Ivy), etc.: 1-6 Tablets/liter water (50-300 ppm AI)

Hardwood Cuttings, Conifers,etc.: 4-10 Tablets/liter water (200-500 ppm AI)

SPRAY DRIP DOWN METHOD ™

Plant Cuttings Then Spray

Plant cuttings. Spray solution on leaves and stems until drip down into media.

Herbaceous Cuttings, Chrysanthemum, Begonia, Dieffenbachia, Heath, Hibiscus, etc.: 1-5 Tablets/liter water (50-250 ppm AI)

For Cuttings Which Take a Long Time to Root, or When Roots of Cuttings are Killed by Root Fungus.

Periodically spray solution on leaves and stems until drip down into media: 1-5 Tablets/liter water (50-250 ppm AI)

TRANSPLANTING

Use solutions to promote root regeneration when transplanting.

Immersion- Absorption Method (treat bare root plants).

Soil Drench Method (treat plants in plug or ball).

Soil Drench Method (treat transplants already planted).

IMMERSION-ABSORPTION METHOD ™ (Bare Root Plants)

Dissolve Tablets in water only. Immerse bare roots in solution. Plant immediately or use before storage.

Immerse roots in solution at 2-3 Tablets/liter water for 10 minutes, or 3-5 Tablets/liter water for 5 minutes. After treating roots optionally spray the leaves lightly using ½ to 1 Tablet/liter water.

SPRAY OR DIP METHODS (Plants in Plug or Ball)

Spray plug, ball or bare roots after taking the plants out of propagation tray, or dip whole plug or ball in solution until fully saturated.

Herbaceous Plants, House and Foliage Plants: 1-3 Tablets/ liter water.

Woody, Ornamental and Forestry Plants: 3-5 Tablets/liter water.

After treating roots optionally spray the leaves lightly using ½ to 1 Tablet/liter water.

SOIL DRENCH METHOD (After Planting)

After planting drench the soil.

House and Foliage Plants: 1-2 Tablets/liter water.

Herbaceous Plants: 2-4 Tablets/liter water.

Woody, Ornamental and Forestry Plants: 4-8 Tablets/liter water.

Use higher rate when soil composition draws water away from the roots.

After treating roots optionally spray the leaves lightly using ½ to 1 Tablet/liter water.

IMPROVE PLANT GROWTH

Use on house, tropical, herbaceous, bedding, flowering plants.

Improve growth and flowering by increasing root mass. Apply at 3-5 week intervals during the active growing season or whenever fertilizing. Apply after harvesting to improve flower regeneration. Drench the soil and or spray foliage. Use 1/10 to 1 Tablet/liter water.

Hardy Ornamental Plants, Deciduous and Evergreen Shrubs and Trees.

Apply at 4-6 week intervals early in the active growing season or whenever fertilizing. Apply 1 or 2 times late in the growing season to reduce winter kill and improve following year growth. Drench the soil and or spray foliage. Use 1/4 to 2 Tablets/liter water.

TO MAKE A SOLUTION OF ANY VOLUME

For many applications dissolve tablets using water only.

Mix a portion of the solution for the same day use. Dispose of un-used portion as described in the 'storage and disposal' statements on this label. Active Ingredients entirely dissolve, in water up to 10 Tablets/liter (500 ppm AI), in water and alcohol to 400 Tablets/liter (20000 ppm AI).

A small amount of visible inert ingredients do not affect results.

Container: Use plastic not metal.

Water: Use clean water preferably distilled or deionized.

Alcohol: Use methyl or isopropyl or ethyl.

Temperature: Dissolve Tablets at 68F-85F.

'Blender' is an electric hand blender (liquefier). Stir at least 1 minute or until dissolved. When using a blender mix an adequate amount of liquid to assure adequate blender action.

THREE EASY STEPS

(1) Decide the concentration in Tablets/liter or ppm AI.

(2) Decide the Working Volume V.

(3) Measure and mix.

MIXING INSTRUCTIONS

0-5 Tablets/liter (0-250 ppm AI): -Dissolve in water. Mix manually or use blender.

5-10 Tablets/liter (250-500 ppm AI): -Dissolve in water. Mix using blender

11-200 Tablets/liter (550-10000 ppm AI): -Make an initial 10000 ppm AI concentration using 50% water+50% alcohol. Dilute with water to make working concentration. Mix using blender. See STEPS TO CALCULATE below.

201-400 Tablets/liter (10050-20000 ppm AI): -Use 25% water+75% alcohol. Mix using blender.

EXAMPLES TO MAKE 1 LITER (1000 ml) SOLUTION

For the following mix manually or use an electric hand blender:

250 ppm AI: mix 5 Tablets in 1000 ml water.

For the following mix using an electric hand blender:

500 ppm AI: mix 10 Tablets in 1000 ml water.

1000 ppm AI: mix 20 Tablets in 50ml water + 50ml alcohol, add water to 1000 ml.

2000 ppm AI: mix 40 Tablets in 100ml water+ 100ml alcohol, add water to 1000 ml.

5000 ppm AI: mix 100 Tab. in 250ml water+ 250ml alcohol, add water to 1000 ml.

8000 ppm AI: mix 160 Tab. in 400ml water+ 400ml alcohol, add water to 1000 ml.

10000 ppm AI: mix 200 Tablets in 500 ml water + 500 ml alcohol.

20000 ppm AI: mix 400 Tablets in 250 ml water + 750 ml alcohol.

TO CALCULATE THE NUMBER OF TABLETS T REQUIRED FOR A GIVEN VOLUME V

Given Working Volume V and ppm AI:

V (Liters)=ml./1000

P=ppm AI /50

The Number of Tablets T= V (Liters) x P

Given Working Volume V and Tablets/liter:

V (Liters) =ml./1000

N = Tablets/liter

The Number of Tablets T =V (Liters) x N

Up to 5 Tablets/liter Water (0-250 ppm AI)

(1) Calculate the Number of Tablets T.
(2) Measure the Working Volume V using water only.
(3) Dissolve Tablets in water; mix manually or using hand blender.

5-10 Tablets/liter Water (300-500 ppm AI)

(1) Calculate the Number of Tablets T.
(2) Measure the Working Volume V using water only.
(3) Dissolve Tablets in water; mix using hand blender.

11-200 Tablets/liter Solution (550-10000 ppm AI)

(1) Calculate the Number of Tablets T.
(2) Calculate the initial mixing volume of water and alcohol (1:1) to dissolve Tablets. Water (ml.) = Number of Tablets T x 2.5 Alcohol (ml.) = Number of Tablets T x 2.5
(3) Measure water and alcohol. Dissolve Tablets in water and alcohol; mix using hand blender.
(4) Dilute with water to the Working Volume V (Liters).

200-400 Tablets/liter Solution (10000-20000 ppm AI)

(1) Calculate the Number of Tablets T.
(2) Measure Working Volume V using 25% water and 75% alcohol.
(3) Dissolve Tablets in water and alcohol; mix using hand blender.

STORAGE AND DISPOSAL

Do not contaminate water, food, or feed by storage and disposal.

Pesticide Storage: Store in a cool dry place. Keep in original container.

Pesticide Disposal: Wastes resulting from this product m ay be disposed of on site or at an approved waste disposal facility.

Container Disposal: Triple rinse (or equivalent). Then offer for recycling, or reconditioning, or puncture and dispose of in a sanitary landfill, or incineration, or, if allowed by state and local authorities, by burning. If burned stay out of smoke.

WARRANTY

It is warranted that this product conforms to the chemical description on the label thereof and is reasonably fit for purposes stated on such label only when used in accordance with the directions under normal use conditions. It is impossible to eliminate all risks inherently associated with the use of this product. Crop injury, ineffectiveness or other unintended consequences may result because of such factors as weather conditions, presence of other materials, or the manner of application all of which is beyond the control of Rhizopon b.v. or Hortus USA Corp. To the extent of the law, Rhizopon or Hortus USA shall not be liable for consequential, special or indirect damages resulting from the use or handling of this product. To the extent of the law, all risks shall be assumed by the buyer. Rhizopon or Hortus USA make no warranties of merchantability or fitness for a particular purpose nor other express or implied warranty except as stated above.

Hortus USA Corp.
PO Box 1956, New York, NY 10113
Master Distributor: Phytotronics
13688 Rider Trail N, Earth City MO 63045 USA.
Phone: 314-770-0717
www.rooting-hormones.com

HORTUS

IBA

Water Soluble

Salts ™ (20%)

Plant Growth Regulator - Rooting Hormone

Propagate New Plants by Rooting Cuttings.
Improve Transplanting.
Promote Root Regeneration.
Improve Plant Growth & Flower Yields.
Build Root Mass.

Use on house, tropical, herbaceous, perennial and foliage plants, hardy ornamental plants, trees & shrubs, evergreen and deciduous plants.
Use in Greenhouses and Nurseries Only

Active Ingredients:
Indole-3-butyric Acid ...20.0%
Other Ingredients....80.0%
Total...100.0%

Contents: 1 kg
(Equal to .2 kg 100% IBA)
EPA Reg #63310-22
EPA Est #63310 HL-001

Distributed by
PHYTOTRONICS, INC.
13688 Rider Trail North
Earth City MO 63045
(314-770-0717)
Registered by
HORTUS USA CORP.
New York NY 10011

Keep Out Of Reach Of Children
CAUTION

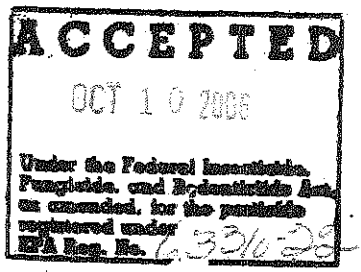
See Additional Precautionary Statements on Back Panel

Use Hortus IBA Water Soluble Salts (20%) to propagate new plants from cuttings. The new plants will have the same characteristics as the parent plant.

Treated cuttings will expect to produce uniform roots all around the basal end of the cuttings; the results are beautiful symmetric plants.

Hortus IBA Water Soluble Salts (20%) dissolve easily in WATER up to 60,000 ppm IBA.

Hortus IBA Water Soluble Salts (20%), are easier to measure accurate concentrations than 100% IBA salts due to percentage formulation.



PRECAUTIONARY STATEMENTS
Hazards to humans and domestic animals.

CAUTION
Causes moderate eye injury. Harmful if inhaled or absorbed through the skin. Avoid contact with eyes, skin, ingestion or inhalation. Avoid breathing dust (vapor or spray mist). Wash thoroughly with soap and water after handling. Remove contaminated clothing and wash before reuse.

PERSONAL PROTECTIVE EQUIPMENT
Applicators and other handlers must wear: long sleeve shirt, long pants, shoes, socks and waterproof gloves, and for exposure in enclosed areas or outdoors, dust/mist filtering respirator. (MSHA/NIOSH approval number prefix TC-21C) or a NIOSH-approved respirator with any R, P or HE filter.

USER SAFETY RECOMMENDATIONS
Users should: Wash hands before eating, drinking, chewing gum, using tobacco or using the toilet. Remove clothing immediately if pesticide gets inside. Then wash thoroughly and put on clean clothing.
Remove PPE immediately after handling this product. Wash the outside of gloves before removing. As soon as possible, wash thoroughly and change into clean clothing.

FIRST AID Classification of Pesticide: Plant Growth Regulator	
If in eyes	> Hold eyes open and rinse slowly with water for 15-20 minutes > Remove contact lenses, if present after the first 5 minutes then continue rinsing eye > Call a poison center or a doctor for further treatment or advise
If on skin or clothing	> Take off contaminated clothing > Rinse skin immediately with plenty of water for 15-20 minutes > Call a poison center or a doctor for further treatment or advise
If swallowed	> Call a poison center or a doctor for further treatment or advise > Have person sip water if able to swallow > Do not induce vomiting unless told to do so by the poison control center or doctor. > Do not give anything by mouth to an unconscious person.
If inhaled	> Move person to fresh air > If person is not breathing call 911 or an ambulance, then give artificial respiration, preferably by mouth to mouth, if possible > Call a poison center or a doctor for further treatment or advise
NOTE TO PHYSICIAN: May cause minor eye irritation which will last a short time. This product does NOT contain any petroleum, caustics or active solvent products	
Have the product container or label with you when you call a poison control center or doctor or going for treatment. You may also call 800-325-3055 or 314-770-0717. 7:30AM-5PM CST Mon-Fri or the National Pesticide Information Center (NPIC) at 1-800-858-7378	

You may also call 800-325-3055 or 314-770-0717 for an MSDS. 7:30AM-5PM CST Mon-Fri

ENVIRONMENTAL HAZARDS
Do not apply directly to water, or to areas where surface water is present or to intertidal areas below the mean high water mark. Do not contaminate water by cleaning of equipment or disposal of equipment wash waters.

DIRECTIONS FOR USE

It is a violation of Federal law to use this product in a manner inconsistent with labeling. For any requirements specific to your State or Tribe, consult the agency responsible.

AGRICULTURAL USE REQUIREMENTS

Use this product in accordance with its labeling and with the Worker Protection Standard, 40 CFR 170. This Standard contains requirements for the protection of agricultural workers on farms, forests nurseries, and greenhouses, and handlers of agricultural pesticides. It contains requirements for training, decontamination, notification, and emergency assistance. It also contains specific instructions and exceptions pertaining to the statements on this labeling about personal protective equipment and restricted-entry intervals.

The requirements in this box only apply to the uses of this product that are covered by the Workers Protection Standard (WPS).

Do not apply this product in a way that will contact workers or other persons, either directly or through drift. Only protected handlers may be in the area during application.

THE RESTRICTED-ENTRY INTERVAL (REI)
FOR THIS PRODUCT IS "0" HOURS.

RATE DETERMINATION

A wide dosage range is indicated for most uses of this product. The ideal rate will vary according to specific plant variety and local growing conditions. To assure compatibility of this product under the specific growing conditions and to determine the dosage rate best suited for your growing conditions. **It is necessary that you test a few plants at varying rates within the specified dosage range prior to large scale operations.** If combined with other plant regulators, test varying rates of each product. If foliar application causes phytotoxicity, try soil applications or decreased rates

Always use the lowest possible concentration of Hortus IBA Water Soluble Salts to produce the desired effect

PREPARING A HORTUS IBA WATER SOLUBLE SALT (20%) SOLUTION

Hortus IBA Water Soluble Salts contain 20% IBA.
When calculating parts per million (ppm) IBA use five times the weight of Hortus IBA Water Soluble Salts (20%) compared to 100% IBA.

Mix a portion of the solution for the same day use.
Dispose of un-used portion as described in the 'storage and disposal' statements on this label.
Container: Use plastic not metal.
Temperature: Dissolve at 68F-85F.
Water: Mix Hortus IBA Water Soluble Salts (20%) with WATER only.
To avoid precipitation problems with the solutions use distilled or demineralized water not tap water. Do not dissolve salts in other solvents.

The following table illustrates typical concentrations from 500 to 10,000 ppm IBA

Final Conc. ppm IBA	Weight of Salts per Liter of Solution (dissolve in WATER)			
	Weight of Hortus IBA Water Soluble Salts (20%) per liter of water		Equivalent weight of 100% IBA	
	mg	gram	mg	gram
100	500	0.5	100	0.1
200	1000	1.0	200	0.2
300	1500	1.5	300	0.3
400	2000	2.0	400	0.4
500	2500	2.5	500	0.5
600	3000	3.0	600	0.6
700	3500	3.5	700	0.7
800	4000	4.0	800	0.8
900	4500	4.5	900	0.9
1,000	5000	5.0	1,000	1.0
5,000	25,000	25.0	5,000	5.0
10,000	50,000	50.0	10,000	10.0

To make a 10,000 ppm IBA stock solution (10,000 mg/liter or **1 percent** stock solution) dissolve **50.0 grams of Hortus IBA Water Soluble Salts** (20%) in **one liter of water.**
To make a one liter (1000 ml) of a 1000 ppm IBA working solution from a 1 percent stock solution add 100 ml of the
1 percent stock solution to 900 ml of water.

PROMOTE ROOTING OF PLANT CUTTINGS

Use to propagate new plants from cutting- from those easy to root to the most difficult to root.

EASY STEPS

1. Take plant cuttings, usually stem cuttings.
2. For woody cuttings usually wound by making a 3/4" notch at basal end.
3. Apply to plant cuttings by convenient methods:

Total Immerse: Completely immerse several cuttings at a time for a few seconds then plant.
Quick Dip: Immerse basal end of cuttings for a few seconds then plant.
Spray Drip Down ™: Plant many cuttings in trays then spray cuttings until drips down.
Immersion Method: Immerse basal end of cuttings for a few hours then plant.

4. Take care of cuttings as appropriate for the plant variety.

Control watering, temperature, humidity, light and other environmental factors. Observe and control external factors such as insects and fungus.
Use the minimum concentration to achieve results. (Excessive concentration may inhibit root formation).

IMMERSION METHOD
Immerse basal end then plant.
Immerse basal end of cuttings approximately 1" in solution for 4 to12 hours. Plant immediately.
Herbaceous and Woody Cuttings, including hard to root cuttings and rootstocks: 50-150 ppm IBA

QUICK DIP METHOD
Immerse basal end then plant.
Immerse basal end of cuttings approximately 1" in solution a few seconds. Plant immediately.
Herbaceous Cuttings, Tropical Plants, House Plants, Roses, etc.: 150-500 ppm IBA
Chrysanthemum: 200-500 ppm IBA.
Softwood Cuttings: 500-1000 ppm IBA.
Hardwood Cuttings: 500-2000 ppm IBA.
Difficult to Root Cuttings: 5000-20000 ppm IBA.

TOTAL IMMERSE METHOD
Total immerse cuttings then plant
Immerse whole cuttings in solution a few seconds. A basket is useful. Plant immediately.
Herbaceous Cuttings, Chrysanthemum, Plumbago, Ivy, Clematis, Delphinium, Lavender, Ficus, Potted Rose Bushes, etc.: 50-250 ppmIBA.
Softwood Cuttings, Buddleia (Butterfly Bush), Hydrangea, Pieris, Hedera (Ivy), etc.: 50-300 ppm IBA.
Hardwood Cuttings, Conifers, etc.: 200-500 ppm IBA.

SPRAY DRIP DOWN METHOD ™
Plant Cuttings Then Spray
Plant cuttings. Spray solution on leaves and stems until drip down into media.
Herbaceous Cuttings, Chrysanthemum, Begonia, Dieffenbachia, Heath, Hibiscus, etc.: 50-250 ppm IBA.

For Cuttings Which Take a Long Time to Root, or When Roots of Cuttings are Killed by Root Fungus: periodically spray solution on leaves and stems until drip down into media: 50-250 ppm IBA

TRANSPLANTING

Use solutions to promote root regeneration when transplanting. **ALWAYS DO SMALL TRIALS PRIOR TO DOING LARGE SCALE PRODUCTION. Use the LOWEST concentration to produce the desired effect.**

IMMERSION-ABSORPTION METHOD ™ (Bare Root Plants)
Immerse bare roots in solution. Plant immediately or use before storage.
Immerse roots in solution at 50 (or less) -150 ppm IBA for 10 minutes, or 150 (or less) - 250 ppm IBA for 5 minutes. After treating roots optionally spray the leaves lightly using 25 (or less) -50 ppm IBA.

SPRAY OR DIP METHODS (Plants in Plug or Ball)
Spray plug, ball or bare roots after taking the plants out of propagation tray, or dip whole plug or ball in solution until fully saturated.
Herbaceous Plants, House and Foliage Plants: 50 (or less) -150 ppm IBA.
Woody, Ornamental and Forestry Plants: 150 (or less)-250 ppm IBA.
After treating roots optionally spray the leaves lightly using 25 (or less)-50 ppm IBA.

SOIL DRENCH METHOD (After Planting)
After planting drench the soil.
House and Foliage Plants: 50 (or less) -100 ppm IBA.
Herbaceous Plants: 50 (or less) -200 ppm IBA.
Woody, Ornamental and Forestry Plants: 200 (or less) -400 ppm IBA.
Use higher rate when soil composition draws water away from the roots. After treating roots optionally spray the leaves lightly using 25 (or less) -50 ppm IBA.

IMPROVE PLANT GROWTH

Use on house, tropical, herbaceous, bedding, flowering plants: Improve growth and flowering by increasing root mass. Apply at 3-5 week intervals during the active growing season or whenever fertilizing. Apply after harvesting to improve flower regeneration. Drench the soil and or spray foliage. Use 5-50 ppm IBA.
Hardy Ornamental Plants, Deciduous and Evergreen Shrubs and Trees: Apply at 4-6 week intervals early in the active growing season or whenever fertilizing. Apply one to two times late in the growing season to reduce winter kill and improve following year growth. Drench the soil and or spray foliage. Use 15-50 ppm IBA.

STORAGE AND DISPOSAL

Do not contaminate water, food, or feed by storage and disposal.
Pesticide Storage: Store in a cool dry place. Keep in original container.
Pesticide Disposal: Wastes resulting from this product may be disposed of on site or at an approved waste disposal facility.
Container Disposal: Triple rinse (or equivalent). Then offer for recycling, or reconditioning, or puncture and dispose of in a sanitary landfill, or incineration, or, if allowed by state and local authorities, by burning. If burned stay out of smoke

WARRANTY

It is warranted that this product conforms to the chemical description on the label thereof and is reasonably fit for purposes stated on such label only when used in accordance with the directions under normal use conditions. It is impossible to eliminate all risks inherently associated with the use of this product. Crop injury, ineffectiveness or other unintended consequences may result because of such factors as weather conditions, presence of other materials, or the manner of application all of which is beyond the control of Rhizopon b.v. or Hortus USA Corp. To the extent of the law, Rhizopon or Hortus USA shall not be liable for consequential, special or indirect damages resulting from the use or handling of this product. To the extent of the law, all risks shall be assumed by the buyer. Rhizopon or Hortus USA make no warranties of merchantability or fitness for a particular purpose nor other express or implied warranty except as stated above.

Hortus USA Corp.,
PO Box 1956, New York, NY 10113
Master Distributor: Phytotronics
13688 Rider Trail N, Earth City MO 63045 USA.
Phone: 314-770-0717
www.rooting-hormones.com

MATERIAL SAFETY DATA SHEET

SECTION 1 Date Prepared: August 15, 2008

Manufacturer: **Rhizopon bv, Rijndik 263A, Hazerswoude, the Netherlands** **31-71-341-5146** **Importer:** **Hortus USA Corp., PO Box 1956 Old Chelsea Sta., New York NY 10113** **Master Distributor:** **Phytotronics Inc., 13688 Rider Tr N, Earth City Mo 63045, 313-770-0717**	**Identity (Product Name):** **RHIZOPON AA #1**

Section II:

IDENTITY INFORMATION

COMPONENT:	CAS:	PERCENT:
Indole-3-butyric acid (IBA) (Formula: C12 H13 N02)	133-32-4	00.1%
Inert Ingredients (proprietary)		99.9%
Total		100%

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Section III:

PHYSICAL/CHEMICAL CHARACTERISTICS

Appearance: Pink odorless powder

Boiling point: N/A — Specific gravity: N/A

Vapor pressure: N/A — Melting point: N/A

Solubility in water: 0% — Evaporation rate: N/A

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Section IV:

FIRE AND EXPLOSION HAZARD DATA

Flash point: N/A.

Extinguishing media: water, multi purpose dry chemical. Special fire fighting procedures: wear pull protective clothing, positive pressure self contained breathing apparatus. Thoroughly wash all equipment after use.

Unusual fire and explosion hazards: none known

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Section V:

REACTIVITY DATA

Stability: stable.

Incompatibility: strong oxidizers.

Hazardous decomposition products: combustion may produce carbon dioxide, nitrogen oxide, and carbon monoxide.

Hazardous polymerization: will not occur.

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Section VI:

HEALTH HAZARD DATA

CAUTION: KEEP OUT OF REACH OF CHILDREN.

FIRST AID Classification of Pesticide: Plant Growth Regulator	
If in eyes	> Hold eyes open and rinse slowly with water for 15-20 minutes > Remove contact lenses, if present after the first 5 minutes then continue rinsing eye > Call a poison center or a doctor for further treatment or advise
If on skin or clothing	> Take off contaminated clothing > Rinse skin immediately with plenty of water for 15-20 minutes > Call a poison center or a doctor for further treatment or advise
If swallowed	> Call a poison center or a doctor for further treatment or advise > Have person sip water if able to swallow > Do not induce vomiting unless told to do so by the poison control center or doctor. > Do not give anything by mouth to an unconscious person.
If inhaled	> Move person to fresh air > If person is not breathing call 911 or an ambulance, then give artificial respiration, preferably by mouth to mouth, if possible > Call a poison center or a doctor for further treatment or advise
NOTE TO PHYSICIAN: May cause minor eye irritation which will last a short time. This product does NOT contain any petroleum, caustics or active solvent products	

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Section VII:

PRECAUTIONS FOR SAFE HANDLING AND USE

Storage and disposal: Store in a cool dry place. Keep in original container. For disposal securely wrap closed container in several layers of newspaper and discard in trash. Do not reuse container.

Environmental hazards: Do not contaminate water when disposing of equipment wash waters. Do not apply directly to water, to areas where surface water is present or intertidal areas below mean high water mark.

CAUTION

Causes moderate eye injury. Harmful if inhaled or absorbed through the skin. Avoid contact with eyes, skin, ingestion or inhalation. Avoid breathing dust (vapor or spray mist). Wash thoroughly with soap and water after handling. Remove contaminated clothing and wash before reuse.

PERSONAL PROTECTIVE EQUIPMENT

Applicators and other handlers must wear: long sleeve shirt, long pants, shoes, socks and waterproof gloves, and for exposure in enclosed areas or outdoors, dust/mist filtering respirator. (MSHA/NIOSH approval number prefix TC-21C) or a NIOSH-approved respirator with any R, P or HE filter.

USER SAFETY RECOMMENDATIONS

Users should: Wash hands before eating, drinking, chewing gum, using tobacco or using the toilet. Remove clothing/PPE immediately if pesticide gets inside. Then wash thoroughly and put on clean clothing.

Remove PPE immediately after handling this product. Wash the outside of gloves before removing. As soon as possible, wash thoroughly and change into clean clothing.

==========

Section VIII:

ADDITIONAL INFORMATION

The information contained herein is furnished without warranty of any kind. Users should consider these data only as supplement to other information gathered by them and must make independent determinations of the suitability and completeness of information from all sources to assure proper use and disposal of these materials and the safety and health of employees and customers.

MATERIAL SAFETY DATA SHEET

SECTION 1 Date Prepared: August 15, 2008

Manufacturer: **Rhizopon bv, Rijndik 263A, Hazerswoude, the Netherlands** **31-71-341-5146** **Importer:** **Hortus USA Corp., PO Box 1956 Old Chelsea Sta., New York NY 10113** **Master Distributor:** **Phytotronics Inc., 13688 Rider Tr N, Earth City Mo 63045, 313-770-0717**	**Identity (Product Name):** **RHIZOPON AA #2**

Section II:

IDENTITY INFORMATION

COMPONENT:	CAS:	PERCENT:
Indole-3-butyric acid (IBA) (Formula: C12 H13 N02)	133-32-4	00.3%
Inert Ingredients (proprietary)		99.7%
Total		100%

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Section III:

PHYSICAL/CHEMICAL CHARACTERISTICS

Appearance: green odorless powder

Boiling point: N/A — Specific gravity: N/A

Vapor pressure: N/A — Melting point: N/A

Solubility in water: 0% — Evaporation rate: N/A

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Section IV:

FIRE AND EXPLOSION HAZARD DATA

Flash point: N/A.

Extinguishing media: water, multi purpose dry chemical. Special fire fighting procedures: wear pull protective clothing, positive pressure self contained breathing apparatus. Thoroughly wash all equipment after use.

Unusual fire and explosion hazards: none known

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Section V:

REACTIVITY DATA

Stability: stable.

Incompatibility: strong oxidizers.

Hazardous decomposition products: combustion may produce carbon dioxide, nitrogen oxide, and carbon monoxide.

Hazardous polymerization: will not occur.

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Section VI:

HEALTH HAZARD DATA

CAUTION: KEEP OUT OF REACH OF CHILDREN.

FIRST AID Classification of Pesticide: Plant Growth Regulator	
If in eyes	> Hold eyes open and rinse slowly with water for 15-20 minutes > Remove contact lenses, if present after the first 5 minutes then continue rinsing eye > Call a poison center or a doctor for further treatment or advise
If on skin or clothing	> Take off contaminated clothing > Rinse skin immediately with plenty of water for 15-20 minutes > Call a poison center or a doctor for further treatment or advise
If swallowed	> Call a poison center or a doctor for further treatment or advise > Have person sip water if able to swallow > Do not induce vomiting unless told to do so by the poison control center or doctor. > Do not give anything by mouth to an unconscious person.
If inhaled	*> Move person to fresh air* > If person is not breathing call 911 or an ambulance, then give artificial respiration, preferably by mouth to mouth, if possible > Call a poison center or a doctor for further treatment or advise
NOTE TO PHYSICIAN: May cause minor eye irritation which will last a short time. This product does NOT contain any petroleum, caustics or active solvent products	

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Section VII:

PRECAUTIONS FOR SAFE HANDLING AND USE

Storage and disposal: Store in a cool dry place. Keep in original container. For disposal securely wrap closed container in several layers of newspaper and discard in trash. Do not reuse container.

Environmental hazards: Do not contaminate water when disposing of equipment wash waters. Do not apply directly to water, to areas where surface water is present or intertidal areas below mean high water mark.

CAUTION

Causes moderate eye injury. Harmful if inhaled or absorbed through the skin. Avoid contact with eyes, skin, ingestion or inhalation. Avoid breathing dust (vapor or spray mist). Wash thoroughly with soap and water after handling. Remove contaminated clothing and wash before reuse.

PERSONAL PROTECTIVE EQUIPMENT

Applicators and other handlers must wear: long sleeve shirt, long pants, shoes, socks and waterproof gloves, and for exposure in enclosed areas or outdoors, dust/mist filtering respirator. (MSHA/NIOSH approval number prefix TC-21C) or a NIOSH-approved respirator with any R, P or HE filter.

USER SAFETY RECOMMENDATIONS

Users should: Wash hands before eating, drinking, chewing gum, using tobacco or using the toilet. Remove clothing/PPE immediately if pesticide gets inside. Then wash thoroughly and put on clean clothing.

Remove PPE immediately after handling this product. Wash the outside of gloves before removing. As soon as possible, wash thoroughly and change into clean clothing.

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Section VIII:

ADDITIONAL INFORMATION

The information contained herein is furnished without warranty of any kind. Users should consider these data only as supplement to other information gathered by them and must make independent determinations of the suitability and completeness of information from all sources to assure proper use and disposal of these materials and the safety and health of employees and customers.

MATERIAL SAFETY DATA SHEET

SECTION 1 Date Prepared: August 15, 2008

Manufacturer: **Rhizopon bv, Rijndik 263A, Hazerswoude, the Netherlands** **31-71-341-5146** **Importer:** **Hortus USA Corp., PO Box 1956 Old Chelsea Sta., New York NY 10113** **Master Distributor:** **Phytotronics Inc., 13688 Rider Tr N, Earth City Mo 63045, 313-770-0717**	**Identity (Product Name):_** **RHIZOPON AA #3**

Section II:

IDENTITY INFORMATION

COMPONENT:	CAS:	PERCENT:
Indole-3-butyric acid (IBA) (Formula: C12 H13 N02)	133-32-4	00.8%
Inert Ingredients (proprietary)		99.2%
Total		100%

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Section III:

PHYSICAL/CHEMICAL CHARACTERISTICS

Appearance: white-gray odorless powder

Boiling point: N/A — Specific gravity: N/A

Vapor pressure: N/A — Melting point: N/A

Solubility in water: 0% — Evaporation rate: N/A

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Section IV:

FIRE AND EXPLOSION HAZARD DATA

Flash point: N/A.

Extinguishing media: water, multi purpose dry chemical. Special fire fighting procedures: wear pull protective clothing, positive pressure self contained breathing apparatus. Thoroughly wash all equipment after use.

Unusual fire and explosion hazards: none known

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Section V:

REACTIVITY DATA

Stability: stable.

Incompatibility: strong oxidizers.

Hazardous decomposition products: combustion may produce carbon dioxide, nitrogen oxide, and carbon monoxide.

Hazardous polymerization: will not occur.

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Section VI:

HEALTH HAZARD DATA

CAUTION: KEEP OUT OF REACH OF CHILDREN.

FIRST AID Classification of Pesticide: Plant Growth Regulator	
If in eyes	> Hold eyes open and rinse slowly with water for 15-20 minutes > Remove contact lenses, if present after the first 5 minutes then continue rinsing eye > Call a poison center or a doctor for further treatment or advise
If on skin or clothing	> Take off contaminated clothing > Rinse skin immediately with plenty of water for 15-20 minutes > Call a poison center or a doctor for further treatment or advise
If swallowed	> Call a poison center or a doctor for further treatment or advise > Have person sip water if able to swallow > Do not induce vomiting unless told to do so by the poison control center or doctor. > Do not give anything by mouth to an unconscious person.
If inhaled	*> Move person to fresh air* > If person is not breathing call 911 or an ambulance, then give artificial respiration, preferably by mouth to mouth, if possible > Call a poison center or a doctor for further treatment or advise
NOTE TO PHYSICIAN: May cause minor eye irritation which will last a short time. This product does NOT contain any petroleum, caustics or active solvent products	

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Section VII:

PRECAUTIONS FOR SAFE HANDLING AND USE

Storage and disposal: Store in a cool dry place. Keep in original container. For disposal securely wrap closed container in several layers of newspaper and discard in trash. Do not reuse container.

Environmental hazards: Do not contaminate water when disposing of equipment wash waters. Do not apply directly to water, to areas where surface water is present or intertidal areas below mean high water mark.

CAUTION

Causes moderate eye injury. Harmful if inhaled or absorbed through the skin. Avoid contact with eyes, skin, ingestion or inhalation. Avoid breathing dust (vapor or spray mist). Wash thoroughly with soap and water after handling. Remove contaminated clothing and wash before reuse.

PERSONAL PROTECTIVE EQUIPMENT

Applicators and other handlers must wear: long sleeve shirt, long pants, shoes, socks and waterproof gloves, and for exposure in enclosed areas or outdoors, dust/mist filtering respirator. (MSHA/NIOSH approval number prefix TC-21C) or a NIOSH-approved respirator with any R, P or HE filter.

USER SAFETY RECOMMENDATIONS

Users should: Wash hands before eating, drinking, chewing gum, using tobacco or using the toilet. Remove clothing/PPE immediately if pesticide gets inside. Then wash thoroughly and put on clean clothing.

Remove PPE immediately after handling this product. Wash the outside of gloves before removing. As soon as possible, wash thoroughly and change into clean clothing.

==========

Section VIII:

ADDITIONAL INFORMATION

The information contained herein is furnished without warranty of any kind. Users should consider these data only as supplement to other information gathered by them and must make independent determinations of the suitability and completeness of information from all sources to assure proper use and disposal of these materials and the safety and health of employees and customers.

MATERIAL SAFETY DATA SHEET

SECTION 1 Date Prepared: August 15, 2008

Manufacturer: **Rhizopon bv, Rijndik 263A, Hazerswoude, the Netherlands** **31-71-341-5146** **Importer:** **Hortus USA Corp., PO Box 1956 Old Chelsea Sta., New York NY 10113** **Master Distributor:** **Phytotronics Inc., 13688 Rider Tr N, Earth City Mo 63045, 313-770-0717**	**Identity (Product Name):** **RHIZOPON AA WATER SOLUBLE TABLETS**

Section II:

IDENTITY INFORMATION

COMPONENT:	CAS:	PERCENT:	MG PER TABLET
Indole-3-butyric acid (IBA) (Formula: C12 H13 N02)	133-32-4	20%	50 mg
Inert ingredients (proprietary)		80%	200 mg
Total per tablet		100%	250 mg

==========

Section III:

PHYSICAL/CHEMICAL CHARACTERISTICS

Appearance: white odorless tablet

Boiling point: N/A

Specific gravity: N/A

Vapor pressure: N/A

Melting point: N/A

Solubility in water: 100%

Evaporation rate: N/A

==========

Section IV:

FIRE AND EXPLOSION HAZARD DATA

Flash point: N/A.

Extinguishing media: water, multi purpose dry chemical. Special fire fighting procedures: wear pull protective clothing, positive pressure self contained breathing apparatus. Thoroughly wash all equipment after use.

Unusual fire and explosion hazards: none known

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Section V:

REACTIVITY DATA

Stability: stable.

Incompatibility: strong oxidizers.

Hazardous decomposition products: combustion may produce carbon dioxide, nitrogen oxide, and carbon monoxide.

Hazardous polymerization: will not occur.

==========

Section VI:

HEALTH HAZARD DATA

CAUTION: KEEP OUT OF REACH OF CHILDREN.

FIRST AID Classification of Pesticide: Plant Growth Regulator	
If in eyes	> Hold eyes open and rinse slowly with water for 15-20 minutes > Remove contact lenses, if present after the first 5 minutes then continue rinsing eye > Call a poison center or a doctor for further treatment or advise
If on skin or clothing	> Take off contaminated clothing > Rinse skin immedialely with plenty of water for 15-20 minutes > Call a poison center or a doctor for further treatment or advise
If swallowed	> Call a poison center or a doctor for further treatment or advise > Have person sip water if able to swallow > Do not induce vomiting unless told to do so by the poison control center or doctor. > Do not give anything by mouth to an unconscious person.
If inhaled	> Move person to fresh air > If person is not breathing call 911 or an ambulance, then give artificial respiration, preferably by mouth to mouth, if possible > Call a poison center or a doctor for further treatment or advise
NOTE TO PHYSICIAN: May cause minor eye irritation which will last a short time. This product does NOT contain any petroleum, caustics or active solvent products	

==========

Section VII:

PRECAUTIONS FOR SAFE HANDLING AND USE

Storage and disposal: Store in a cool dry place. Keep in original container. For disposal securely wrap closed container in several layers of newspaper and discard in trash. Do not reuse container.

Environmental hazards: Do not contaminate water when disposing of equipment wash waters. Do not apply directly to water, to areas where surface water is present or intertidal areas below mean high water mark.

CAUTION

Causes moderate eye injury. Harmful if inhaled or absorbed through the skin. Avoid contact with eyes, skin, ingestion or inhalation. Avoid breathing dust (vapor or spray mist). Wash thoroughly with soap and water after handling. Remove contaminated clothing and wash before reuse.

PERSONAL PROTECTIVE EQUIPMENT

Applicators and other handlers must wear: long sleeve shirt, long pants, shoes, socks and waterproof gloves, and for exposure in enclosed areas or outdoors, dust/mist filtering respirator. (MSHA/NIOSH approval number prefix TC-21C) or a NIOSH-approved respirator with any R, P or HE filter.

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SECTION 1 Date Prepared: August 15, 2008

Manufacturer: **Rhizopon bv, Rijndik 263A, Hazerswoude, the Netherlands** **31-71-341-5146** **Importer:** **Hortus USA Corp., PO Box 1956 Old Chelsea Sta., New York NY 10113** **Master Distributor:** **Phytotronics Inc., 13688 Rider Tr N, Earth City Mo 63045, 313-770-0717**	**Identity (Product Name):** **HORTUS IBA WATER SOLUBLE SALTS**

Section II:

IDENTITY INFORMATION

COMPONENT:	CAS:	PERCENT:
Indole-3-butyric acid (IBA) (Formula: C12 H13 N02)	133-32-4	20%
Inert ingredients (proprietary)		80%
Total		100%

==========

Section III:

PHYSICAL/CHEMICAL CHARACTERISTICS

Appearance: white odorless tablet

Boiling point: N/A — Specific gravity: N/A

Vapor pressure: N/A — Melting point: N/A

Solubility in water: 100% — Evaporation rate: N/A

==========

Section IV:

FIRE AND EXPLOSION HAZARD DATA

Flash point: N/A.

Extinguishing media: water, multi purpose dry chemical. Special fire fighting procedures: wear pull protective clothing, positive pressure self contained breathing apparatus. Thoroughly wash all equipment after use.

Unusual fire and explosion hazards: none known

==========

Section V:

REACTIVITY DATA

Stability: stable.

Incompatibility: strong oxidizers.

Hazardous decomposition products: combustion may produce carbon dioxide, nitrogen oxide, and carbon monoxide.

Hazardous polymerization: will not occur.

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Section VI:

HEALTH HAZARD DATA

CAUTION: KEEP OUT OF REACH OF CHILDREN.

FIRST AID Classification of Pesticide: Plant Growth Regulator	
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Adventitious Root Formation In Cuttings

Edited by
TIM D. DAVIS
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NARENDRA SANKHLA

Advances in Plant Sciences Series
VOLUME 2
Theodore R. Dudley, Ph.D., General Editor

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CHAPTER 9

Auxin Metabolism During Adventitious Rooting

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Additional key words: auxin biosynthesis, auxin catabolism, auxin conjugates, basic peroxidase, developmental physiology.
Abbreviations: 2,4-D, 2,4-dichlorophenoxyacetic acid; IAA, indolyl-3-acetic acid; 4-Cl-IAA, 4-chloroindolyl-3-acetic acid; IAc, indolyl-3-acetaldehyde; IAcrA, indolyl-3-acrylic acid; IAN, indolyl-3-acetonitrile; IAox, indolyl-3-acetaldoxime; IBA, indolyl-3-butyric acid; IPyA, indolyl-3-pyruvic acid; NAA, naphthylacetic acid; TNH_2, tryptamine; Tol, tryptophol (indolyl-3-ethanol); Try, L-tryptophan.

INTRODUCTION

The relatively specific root promoting properties of applied natural and synthetic auxins lead us to believe that these substances play some crucial roles in the processes of rooting by cuttings. A high level of endogenous auxin has been causally related to the initiation of adventitious root primordia. However, none of the recent book chapters dealing with auxin metabolism in relation to rooting (Gurumurti et al. 1984, Haissig 1986, Jarvis 1986, Gaspar and Coumans 1987) have considered auxin as the sole determinant. As will be seen from the literature below, there are few available data relating changes in endogenous auxin level to a cutting's ability to initiate roots. For example, no data have indicated an immediate increase in the level of endogenous natural auxin after an application of root inducing natural or synthetic auxin. Nor is there direct evidence that a synthetic auxin might

substitute for a natural one in cells. The physiology of rooting thus must be disembodied from prejudgements; the role of auxin should be reconsidered in view of actually demonstrated developmental physiology and hormonology.

First, in certain species of cuttings, rooting has been described to occur in at least six successive interdependent phases: induction (the period preceding cell division); transverse first division of pericycle cell(s); longitudinal first divisions of daughter cells; continued cell divisions without an increase in gross volume of the meristematic cluster(s); volume increase of cell cluster(s) by cell expansion; and root protrusion (Mitsuhashi-Kato et al. 1978ab, Gaspar 1981). Each of these anatomical phases probably has a specific associated physiology. The need for auxin in each phase is not known but may be different because at least two different phases of sensitivity to IAA are known (Imaseki 1985). Second, it is now becoming clear (Trewavas and Cleland 1983) that sensitivity to growth substances, not concentrations of growth substances, may be the limiting factor in development. If we are to understand the relations between plant hormones and the control of plant development, we must be willing to consider all options, whether they be changes in hormone concentration, changes in sensitivity to hormones, or a combination of these. Third, characterization of the phases of rooting has most often come from analyses of the whole cutting including the leaves. Such analyses produced some types of valid information. For example, aspects of the physiological state characterizing induced or non-induced plants can be simultaneously obtained in all plant parts (Thorpe et al. 1978) as a result of rapid inter-organ communication (Gaspar et al. 1985, Penel et al. 1985). It must be kept in mind, however, that further refinement of techniques is still to be desired for measurements of biochemical changes in the precise tissues and cells that form roots.

The present chapter reviews the literature concerning variation of the levels of endogenous auxins and auxin conjugates in the course of adventitious rooting, including background information relative to anabolism, catabolism, and conjugation of auxins.

NATURAL AUXINS AND THEIR NATURAL CONJUGATES

Biosynthesis of Auxins

The following naturally occurring auxins are known in higher plants: IAA, 4-Cl-IAA, and IAcrA. Their biosynthetic pathways are briefly as follows.

Indolyl-3-acetic acid

IAA is generally regarded as the major auxin, perhaps universally found in higher plants. Other indolic compounds originating from metabolism of IAA have been identified in some plants. Numerous publications about the biosynthesis of IAA indicated that Try is the universal primary precursor of IAA (Sembner et al. 1980). Three main biosynthetic pathways lead from Try to IAA as shown from many biosynthetic studies in various taxa of higher plants (Fig. 1).

Pathway 1. In the IPyA biosynthetic pathway for IAA, Try is first transaminated to IPyA by a Try aminotransferase (EC 2.6.1.27, Forest and Wightman 1972), which is widely distributed in higher plants (Truelsen 1973). IPyA is a labile compound susceptible to oxidation and acids; therefore, its presence as a native compound in plants is difficult to establish. In the second step, IPyA is decarboxylated to IAc (Moore and Shaner 1968, Rajagopal 1968, Gibson et al. 1972b, Purves and Brown 1978). The oxidation of IAc to IAA can be catalyzed by two different enzymes, depending on the species: 1) an NAD-dependent indolylacetaldehyde dehydrogenase in mung bean [*Vigna radiata* (L.) R. Wilczek] seedlings (Wightman and Cohen 1968); or 2) an NAD-independent (using O_2 as acceptor) IAc oxidase (EC 1.2.3.7) in *Avena* coleoptile, *Pisum* spp., and *Nicotiana* spp. (Wightman and Cohen 1968, Rajagopal 1971, Liu et al. 1978, Miyata et al. 1981).

Pathway 2. A Try decarboxylase (EC 4.1.1.28) first decarboxylates Try to TNH_2 (Sherwin 1970, Gibson et al. 1972a) that an amine oxidase (EC 1.4.3.) then converts to IAc (Lantican and Muir 1967, Muir and Lantican 1968, Sherwin and Purves 1969). TNH_2 is apparently present in many families of plants (Schneider et al. 1972, Smith 1977). In some plants, e.g. mung bean, IAc can be reversibly reduced to Tol by an NAD-dependent alcohol dehydrogenase (EC 1.1.1.1, Wightman and Cohen

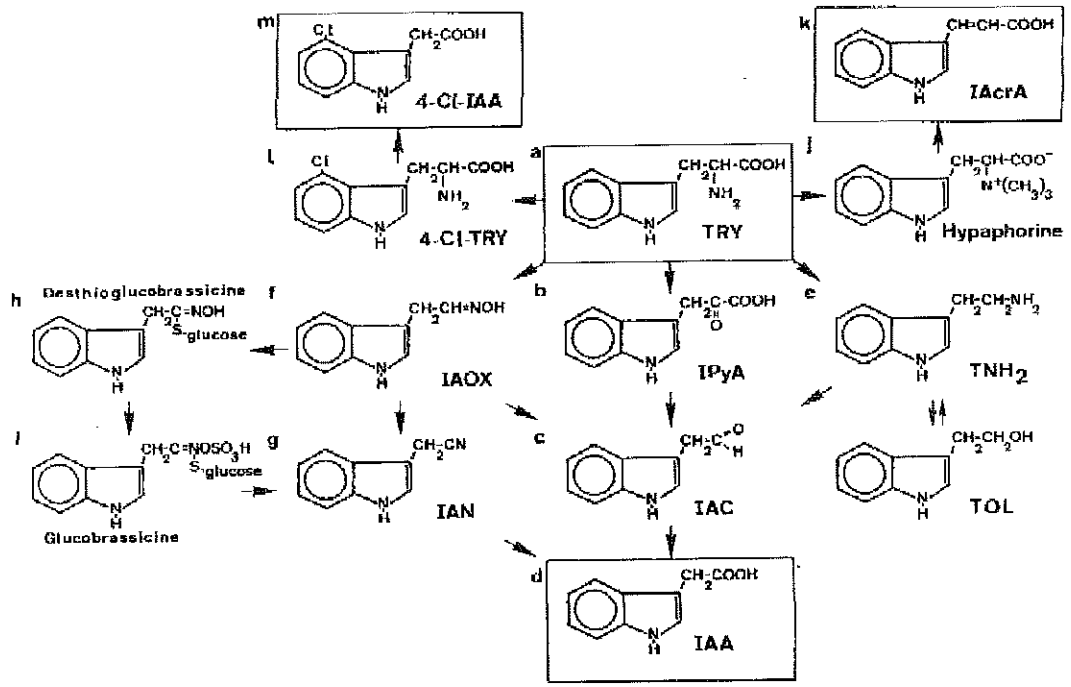

Figure 1. Schematic pathways for biosynthesis of indole auxins. The routes a-b-c-d, a-e-c-d, a-f-c-d, and a-f-g-d lead to indolyl-3-acetic acid in most higher plants. The route a-f-h-l-g-d is typical of the Brassicaceae family. The pathway a-j-k has been established for *Vicia lens (Lens culinaris)*. The pathway a-l-m is restricted to seeds of the Viciae tribe.

1968). An alcohol oxidase (EC 1.1.3.13), which catalyses the irreversible oxidation of Tol to IAc and H_2O_2, was demonstrated in cucumber (*Cucumis sativus* L.) shoots (Vickery and Purves 1972). Tol is assumed to be an important storage product involved in the regulation of IAA biosynthesis equally in plants using pathways 1 or 2 (Percival et al. 1973).

Pathway 3. In this pathway, IAox is formed from Try. Two possible sub-pathways lead from IAox: 1) hydrolysis of IAox may lead to IAc (Rajagopal and Larsen 1972) which is then oxidized to IAA or reduced to Tol; or 2) IAox may be converted by an indoleacetaldoxime dehydratase (EC 4.2.1.29) to IAN, which a nitrilase (EC 3.5.5.1) hydrolyzes to IAA. This second subpathway seems more general (Libbert et al. 1970, Schneider and Wightman 1974). Nitrilase seems to be limited to plant families such as Brassicaceae, Poaceae, and Musaceae (Mahadevan and Thimann 1964, Thimann and Mahadevan 1964). In Brassicaceae an ancillary pathway may convert IAox to the glucosinolates desthioglucobrassicin and glucobrassicin, the latter of which is converted to IAN by myrosinase (EC 3.2.3.1). The physiological significance of this pathway is uncertain (Schraudolf and Weber 1969).

4-Chloroindolyl-3-acetic acid

4-Cl-IAA was identified in immature seeds of some Fabaceae (Marumo et al. 1968, Hofinger and Böttger 1979, Engvild et al. 1981). This compound exhibits strong auxin activity (Marumo et al. 1974), but its physiological significance remains unclear. 4-Cl-IAA is probably specific to the Viciae tribe, although its distribution has not been systematically investigated. It could be involved in seed physiology. 4-Cl-IAA and its methyl ester (Hofinger and Böttger 1979, Engvild et al. 1981), and D-4-chlorotryptophan derivatives (Marumo and Hattori 1970) were isolated from immature seeds of some Fabaceae of the Viciae tribe. 4-Cl-IAA may be formed from D-4-chlorotryptophan but no biosynthetic research has been published.

Indolyl-3-acrylic acid

IAcrA was found in lentil (*Lens culinaris* Med.) seedlings and shown to exhibit auxin activity (Hofinger 1969, Darimont et al. 1971) but its distribution in the plants was not examined nor is its physiological role established. IAcrA was shown to be formed *in vitro* and *in vivo* from Try through hypaphorin (N-trimethyl L-tryptophan (Kutacek et al. 1971, Hofinger et al. 1975)). Hypaphorin occurs naturally in some plants of the Fabaceae family (ref. in Hofinger et al. 1975).

Conjugation of Auxins

Auxins are often covalently bonded (i.e. conjugated) through their carboxyl group to low molecular weight compounds. The resulting conjugates are divided into two categories, depending upon whether they are formed through an amide link with an amino acid (e.g. indolyl-3-acetylaspartate) or an ester link with a sugar or inositol (e.g. indolyl-3-acetylglucose).

Amide conjugates

Indolyl-3-acetylaspartate was the first described major conjugate of IAA (Andreae and Good 1955). Its enzymatic formation can be induced by auxin treatment in all plants studied (Mollan et al. 1972, Epstein and Lavee 1977). It is also widely distributed as a natural compound in untreated plants (Klämbt 1960, Row et al. 1961). The formation of indolyl-3-acetylaspartate needs a lag period during which the synthesis of the responsible enzyme must be induced. This induction may only be realized by auxin treatment (Venis 1972). Light stimulates formation of this conjugate (Muir 1972). From a physiological standpoint, indolyl-3-acetylaspartate is regarded as a detoxification product of exogenous auxins (Zenk 1964, Andreae 1967). It might also be involved in the regulation of the level of the native auxins, because of a reversible conjugation (Feung et al. 1977).

4-Chloroindolyl-3-acetylaspartate methyl ester has been isolated from immature seeds of *Pisum* spp. (Hattori and Marumo 1972). Other amino acid (glycine, alanine, valine, and glutamic acid) conjugates of IAA were found after IAA treatment of *Parthenocissus* spp. callus (Feung et al. 1976).

IAA can also be linked by an amide bond to higher molecular weight compounds, e.g. proteins (Schneider and Wightman 1974, Percival and Bandurski 1976).

Ester conjugates

Ester conjugates of IAA with various sugars [arabinose in maize (*Zea mays* L.) (Steward and Shantz 1959)] and rhamnose in *Peltophorium* spp. (Ganguly et al. 1974)] have been isolated, but glucose is the most common sugar conjugated to IAA (Klämbt 1961, Zenk 1961, 1964, Keglevic and Pokorny 1969, Davies 1972). The formation of IAA-glucose does not need a lag phase and is thus considered to be a rapid detoxification mechanism (Zenk 1964).

Maize kernels are particularly rich in ester conjugates of IAA. Three isomeric glucosyl esters (2′, 4′, and 6′ isomers) have been isolated (Ehmann 1974). In this material a special group of IAA conjugates was isolated which contained the polyol inositol. One inositol molecule may bind one, two, or three IAA molecules (Ehmann and Bandurski 1974). An additional sugar (arabinose, galactose) can be bound by the inositol moiety of IAA-inositol (Ueda and Bandurski 1974). Physiologically, the inositol derivatives of IAA might be storage forms of IAA in desiccated kernels (Ueda and Bandurski 1974) and a transport form of IAA (its transport rate is 1000 fold that of IAA). They could also be involved in the light control of native IAA levels by conjugating or releasing IAA (Bandurski et al. 1977).

Catabolism of Auxins

IAA is a labile compound that may be degraded either chemically by factors such as acidic pH, heat, light, oxidants, peroxides, or heavy metals; or enzymatically by certain metalloproteins. The so-called IAA-oxidase system, functioning with or without H_2O_2, manganese, and/or monophenols as cofactors, is now believed to consist of one or a group of isoperoxidases (EC 1.11.1.7). Any type of peroxidase is able to destroy IAA *in vitro* but *in vivo* only basic isoperoxidases (high isoelectric point, migrating to a cathode) are thought to be IAA-oxidases (Gaspar et al. 1982).

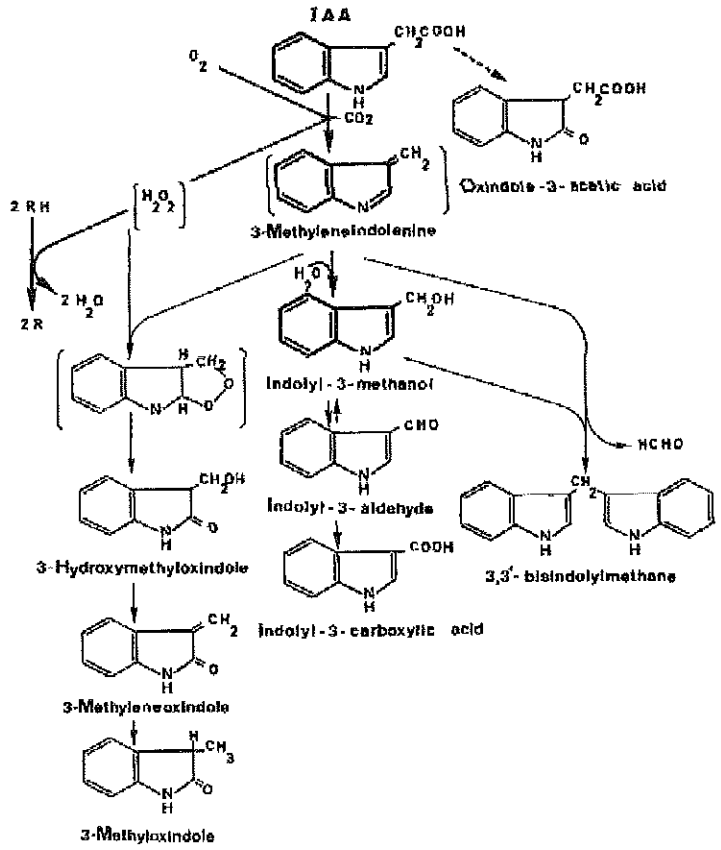

Figure 2. Schematic pathways for (per)oxidative degradation of indolyl-3-acetic acid (IAA). Reactions occurring in the presence of phenolic compounds (RH) have been accentuated with heavy lines. Substances within brackets have not been directly identified. [Adapted from Grambow and Langenbeck-Schwich (1983)]

Depending upon physical and biochemical conditions, IAA can be degraded by peroxidase through different pathways leading to 3-methyleneoxindole or indolyl-3-aldehyde as the main final oxidation products (BeMiller and Colilla 1982, Grambow and Langenbeck-Schwich 1983). Oxindole-3-acetic acid was also identified as a catabolic product of IAA. A methyleneindolenine intermediate probably is the common precursor for the formation of indolyl-3-methanol or 3-hydroxymethyloxindole (Fig. 2). H_2O_2 is produced during IAA decarboxylation as a result of oxidase action. Indolyl-3-methanol is virtually the only product of IAA metabolism if H_2O_2 is consumed by peroxidase action in the presence of phenolic compounds. Indolyl-3-methanol can be reversibly oxidized to indolyl-3-aldehyde, a natural compound in some plants, which itself can be irreversibly oxidized to indolyl-3-carboxylic acid. At a low concentration of phenols, however, excess H_2O_2 is consumed by reaction with the methyleneindolenine intermediate, leading to 3-hydroxymethyloxindole and 3-methyleneoxindole, which can be reduced to 3-methyloxindole by a 3-methyleneoxindole reductase. In biological systems this reaction may function to remove "excess" H_2O_2, thus giving detoxifying properties to the IAA-phenolic co-oxidizing system. As a result, endogenous and exogenous phenolic compounds might indirectly influence rooting through auxin metabolism. For further discussion of auxin metabolizing enzymes, see Chapter 7 by Bhattacharya.

The question of whether auxins are active by themselves or become active only through their metabolism remains unanswered. In particular, methyleneoxindole has been found to exhibit a strong auxinic activity (up to 10-fold compared to IAA (Tuli and Moyed 1969, Hofinger et al. 1980, Bhattacharya et al. 1986) but the reason for this extra activity is unclear (Evans and Ray 1972,

Marumo et al. 1974). IAA contamination could not explain an activity 10-fold higher than IAA; contrariwise, any absence of activity might be due to enzymatic destruction of 3-methyleneoxindole. In relation to the foregoing, we have found methyleneoxindole in the β-inhibitor fraction of lentil roots. Physical or chemical treatment which enhanced auxin destruction *in situ* caused root growth inhibition that corresponded with increasing activity of this β-inhibitor fraction (Gaspar 1973).

ENDOGENOUS AUXIN LEVELS AND ROOTING

Changes in Free IAA-Like Substances

Pioneering research has suggested that the number of roots initiated per cutting may be a function of the amount of auxin-like substances in the regeneration zone (Hemberg 1954, Bastin 1966). In a number of cases, endogenous auxin content has been reported to increase in the base of cuttings during the root inducing period (Blahova 1969, Michniewicz and Kriesel 1970). A relation has been shown between the endogenous acidic auxins and the rooting response to added root promoting substances (Odom and Carpenter 1965), which suggested that optimum levels of auxin were required. A reduced level of auxin has been implicated in the failure of rooting in a number of species of plant cuttings (Cooper 1935, Smith and Wareing 1972ab). However, in other cases auxin levels did not appear to be limiting (Biran and Halevy 1973, Greenwood et al. 1976). For example, Stoltz (1968) found that in Chrysanthemum spp. endogenous auxin concentration did not positively correlate with rooting. In contrast, the number of lateral primordia arising from roots of *Pisum sativum* increased significantly after decapitation; however, a change in the level of IAA was not detected (Böttger 1978). Further complexities were introduced by the observation that, in some easy-to-root cuttings, the endogenous levels of auxin decreased appreciably over the course of rooting (Bose et al. 1973). In another test, auxins in extract fractions that co-chromatographed with IAA were quantified by bioassays at 24 h intervals after pruning *Quercus rubra* seedlings (Carlson and Larson 1977). Auxin activity sharply increased in the first 24 h and then decreased to prepruning levels. New laterals appeared 4–5 d after pruning.

Despite the development of refined techniques for the identification and estimation of auxins, relatively little progress beyond the foregoing observations has been made. By means of gas chromatography, Brunner (1978) identified IBA and IAA in hypocotyl cuttings of *Phaseolus vulgaris* L. In the control cuttings and all the auxin treated cuttings, a distinct increase in the contents of IAA and IBA became evident in the region of root regeneration in the first 24 h. With progressing regeneration, levels of the auxins decreased in all cases. Nakano et al. (1980) also showed that auxin activity at the base of the cuttings diminished during propagation and was lower in well-rooted than in unrooted cuttings. They assumed that the auxin may have been metabolized before root differentiation.

Seasonal changes in hormone levels have been determined in stem cuttings of the easy-to-root *Rhododendron ponticum* L. and the difficult-to-root *Rhododendron* 'Britannia'. The amount of IAA (and cytokinin) in the stem tissue of both groups was similar, with little seasonal variation in either (Wu and Barnes 1981).

Tréfois and Brunner (1982) found a good positive correlation between endogenous auxin-like activity in *Prunus* spp. cuttings and percent of rooting of cuttings only in instances where auxin level was initially high at the time of auxin treatment. There was no effect of endogenous auxin treatment on rooting when the endogenous auxin level was low at the time cuttings were prepared.

Dunberg et al. (1981) found that IAA content at the bases of IBA treated cuttings of *Pinus sylvestris* L. was three times higher than in untreated cuttings. They did not find differences in IAA metabolism or transport between the treated and untreated cuttings, and concluded that applied IBA was converted to IAA by the cuttings. The foregoing results were confirmed by Epstein and Lavee (1984) in olive (*Olea europaea* L.) and grape (*Vitis vinifera* L.) cuttings through the use of radioactive IBA. Using a solid phase enzyme immunoassay, Weigel et al. (1984) have found a large increase of auxin in the apical parts of *Chrysanthemum* spp. shoots after removing them as terminal stem cuttings from stock plants. Auxin level remained high until about when roots were formed. Synthesis of auxin probably occurred because the total amount increased, not only free auxin derived from bound

forms. However, basipetal transport of IAA would have been arrested at the cut end of the cutting, which may have lead to an endogenous, basal accumulation. In this system, prolonged high irradiance of stock plants (40 $W \cdot m^{-2}$) delayed an IAA increase in the cuttings and concomitantly decreased the number of roots per cutting compared to controls (4.5 $W \cdot m^{-2}$). However, root growth as determined by measuring root length or fresh weight were not affected in the latter test. A distinct relation was found between IAA content of stock plants at the time when cuttings were taken and the number of roots formed by the cuttings 20 d later.

Similarly, rooting percentage correlated positively with IAA concentrations in *Cotinus coggygria* Scop. cuttings (Blakesley et al. 1985a): IAA concentration in stem tissue of spring cuttings (which rooted well) was approximately 10 times the concentration in autumn cuttings (which rooted poorly). The concentration of IAA fell markedly after excision and before root appearance (Blakesley et al. 1985a). A transient increase in the concentration of IAA was also discovered in *Phaseolus aureus* hypocotyl cuttings, where root initiation occurs during the first 15 h (Blakesley et al. 1985b).

Changes in Auxin Conjugates

Good rooting of cuttings has sometimes been related to the endogenous level of auxin conjugates. For example, the concentration of IAA esters in poor-rooting autumn *Cotinus* spp. cuttings was 10–20 times higher than that of good-rooting spring cuttings (Blakesley et al. 1985c). Thus, the ratio of free to total IAA was much higher in spring than in autumn cuttings.

In stem cuttings of the easy-to-root *Rhododendron ponticum* and the difficult-to-root *Rhododendron* 'Britannia', Wu and Barnes (1981) measured the highest levels of free and conjugated IAA in summer. However, the content of conjugated IAA decreased between summer and autumn much more in *R.* 'Britannia' than in *R. ponticum*; free IAA on the contrary decreased much more in *R. ponticum*. In another study, terminal stem cuttings of *Chrysanthemum morifolium* Ramat. accumulated both IAA and ester IAA immediately after their removal, compared to levels in stock plants, with a subsequent decline. The maximum total IAA level was reached when the first roots were seen (Weigel et al. 1984). Collet and Le (1987) recently showed that best rooting (rate of rooting, number of roots, absence of callus) of microcuttings of *Rosa* spp. and *Malus* spp. shoots that were produced *in vitro* was achieved by a brief pretreatment of the cut ends with a high concentration of IAA. In the foregoing study, prior to rooting, there was first an accumulation of IAA in the rooting zone, followed by the rapid disappearance of IAA and the appearance of IAA conjugates (IAA-glucosyl, proved by hydrolysis by β-glucosidase). This kind of physiological metabolism-mobilization of "active" compounds was compared by Collet and Le (1987) with the previously demonstrated transformation of radioactively labeled IBA into IAA in the cuttings of *Vitis* spp. and *Olea* spp. (Epstein and Lavee 1984).

In summary, literature dealing with the variation of free and bound auxins in relation to adventitious rooting is very confusing. This confusion has resulted because it has proven difficult to identify the cells which ultimately generate primordia in cuttings and tissue cultures. Frequently, anatomical observations have not been reported in support of experimental results and, understandably so, because primordium initiation is often asynchronous. Another reason is that the physiological state of the donor plants from which cuttings originated has not often been considered. Several strong themes, however, can be gleaned from the literature: 1) there is a good positive correlation between the endogenous free auxin content and the percent of rooting when auxin level is high at the time cuttings are made; 2) free auxin increases in the rooting zone prior to rooting; and 3) the rapid decrease in free IAA level immediately preceding root initiation coincides with the formation of IAA conjugates.

ENZYMIC IAA OXIDATION AND ROOTING

In this section we have excluded papers dealing with changes in IAA-oxidase activity in relation to rooting because this subject is reviewed elsewhere in the present book (see Chapter 7 by Bhattacharya). This section considers only changes that we have observed in peroxidases themselves in the course of rooting.

Physicochemical studies of peroxidases, and our own physiological investigations relating types and activities of peroxidases to growth and morphogenetic processes, have implicated the basic (cationic) isoperoxidases in *in vivo* auxin catabolism (Gaspar et al. 1982) and regulation of endogenous auxin level. Therefore, we have investigated changes in total peroxidase activity and in the isoperoxidase spectrum during the course of rooting (and flowering) by different plants and explants cultured *in vitro* (Quoirin et al. 1974, Gaspar and Van Hoof 1976, Van Hoof and Gaspar 1976, Gaspar et al. 1977b, Thorpe et al. 1978, Druart et al. 1982, Moncousin and Gaspar 1983).

In summary, our observations indicated that total peroxidase activity during rooting and flowering undergo inverse variations (Fig. 3B and D): rooting occurs after the cutting has reached and passed a peak of maximum peroxidase activity, whereas flowering begins after the plant has passed a point of minimum peroxidase activity (Gaspar 1981). The activity or number of acidic isoperoxidases, or both, increases continuously during the course of both processes, indicating that the total peroxidase activity variations are due solely to the basic isoenzymes. The data are characteristic of cuttings from all species analyzed and also correspond relatively well to results from similar investigations on rooting and flowering by others, even though some discrepancies might appear on first examination of the data (Gaspar 1981). Only a few authors (Haissig 1971, Gaspar et al. 1977a, Mitsuhashi-Kato et al. 1978ab) have distinguished an inductive or preparatory phase (no discernable morphological nor histological changes) from an initiative phase (first cell divisions organizing the root primordia), i.e. before phases of root elongation and growth. The physiological necessity of an inductive phase for flowering is, however, generally recognized. In addition, explants (cuttings) may have undergone their inductive phase of rooting or flowering, which quantitatively and qualitatively modified peroxidases, while still attached to the donor plants. In these instances (Fig. 3A and C), peroxidase changes characterizing the initiative phase may take place directly after placing the

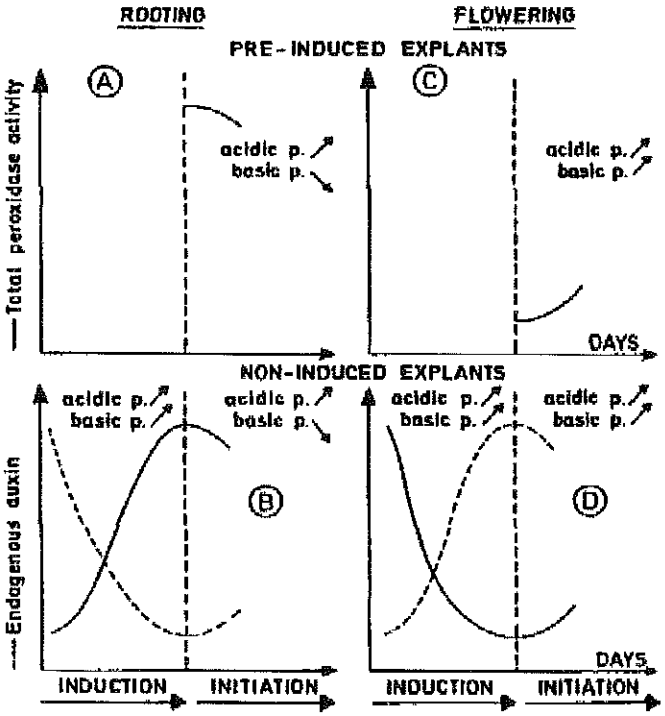

Figure 3. Schematic representation of variations in soluble peroxidase activity (——) and auxin concentration (----) during the induction and initiation phases of rooting (A, B) and flowering (C, D) by preinduced (A, C) and non-preinduced (B, D) explants or plants. The arrows accompanying acidic and basic per(oxidases) indicate an increase or decrease during induction and initiation phases. [Adapted from Gaspar et al. (1982)]

explants in favorable conditions. With regard to endogenous auxin levels, inverse variations in basic isoperoxidases during the inductive and initiative phases of rooting and flowering would also result in inverse variations of auxin content (Fig. 3). Evidence for this hypothesis in flowering has been discussed elsewhere (Gaspar 1981, Gaspar et al. 1985) and seems to apply equally to rooting as described above.

The apparent antagonism that we have described between rooting and flowering have been demonstrated in other tests. For example, antagonistic effects of rooting on the ability of plants to flower were convincingly demonstrated in several species (Miginiac 1971, Sotta and Miginiac 1975, Bismuth et al. 1979), which may explain the promotive effect of root removal on flowering (Krekule and Seidlova 1973) and the reverse (Deletang 1973). The existence of such correlations together with our observations that the biochemical events associated with rooting and flowering characterize the whole plant, cutting, or explant, suggest pronounced hormonal control of these physiological processes.

CONCLUSION

Our analysis of the literature has identified new opportunities and approaches for future research. Past rooting research has somewhat superficially or simplistically studied influences of auxin anabolism and catabolism, in the absence of a needed anatomical or developmental focus. However, it now seems that there are no simple relations between auxin levels in cuttings and rooting. Thus, future research should wherever possible stress auxin anabolism and catabolism in those tissues and cells that initiate root primordia. In addition, data should be interpreted in the context of differential cellular sensitivity to auxin, about which much must be learned.

In terms of the whole cutting, a brief period of metabolic flux precedes rooting. During this period cuttings have a high level of activity of basic (i.e. IAA-oxidase) peroxidases and a low level of auxin, compared to the period of primordium initiation (Gaspar 1981, 1988). However, much additional research is needed in order to determine whether these peroxidase and auxin relations apply equally to preprimordial (target) cells and, if so, whether they directly relate to primordium initiation. There are also other needs for cellular level research concerned with auxin-rooting relations. For example, recent studies indicate that the target cells can be identified in some organs (Chriqui 1985). Exogenous IAA induces rooting from such target cells, starting with initiation of DNA synthesis in G_1 cells. Simultaneously, the G_2 cells enter mitosis. However, the exact relations between endogenous auxin and nucleic acid metabolism in the target cells is poorly understood. In comparison, more is known about teratological growth resulting from treatment with high dosages of 2,4-D and NAA (Nougaréde et al. 1985).

Studies of auxin anabolism and catabolism will be the most meaningful if the degree of cellular sensitivity to auxin is also known. Studies of membrane-based auxin binding protein may help to unravel the cause of differential cellular sensitivity to auxin. For example, a recent study indicated that auxin and cytokinin controlled the presence or absence of auxin binding protein, which was positively correlated with rooting ability (Maan et al. 1985).

Genetic transformation is a sophisticated cellular approach to studying auxin-rooting relations. Auxin biosynthesizing genes from the T-DNA of the bacterium *Agrobacterium* (Liu and Kado 1979) can be inserted into the genome of higher plant cells (Chilton et al. 1982), which then become autonomous for auxin (Black et al. 1986). Such transformation of higher plant tissues by *Agrobacterium rhizogenes* may result in proliferation of adventitious roots ("hairy root" disease, see Chapter 20 by Strobel and Nachmias). One study has shown that the "hairy root" characteristic was stable during plantlet regeneration and associated with significant modifications of isoperoxidases (Benvenuto et al. 1983). Further studies that use *Agrobacterium* transformation may help reveal how variations in peroxidases and auxin metabolism modify cellular differentiation and result in rooting.

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CHAPTER 10

Chemicals and Formulations Used to Promote Adventitious Rooting

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Additional key words: auxin(s), propagation, cuttings, chemical rooting formulations.
Abbreviations and chemical names used: CO, carbon monoxide; C_2H_2, acetylene; C_2H_4, ethylene; C_3H_6, propylene; 2,4-D, 2,4-dichlorophenoxyacetic acid; DMF, dimethyl formamide; ethazol, 5-ethoxy-3-(trichloromethyl)-1,2,4-thiadiazole; IAA, indole-3-acetic acid; IBA, indole-3-butyric acid; IPrA, indole-3-propionic acid; K-salt, potassium salt; NAA, α-naphthaleneacetic acid; β-NAA, β-naphthaleneacetic acid; NAM, α-naphthaleneacetamide; NaOH, sodium hydroxide; NaOCl, sodium hypochlorite; NP-IBA, N-phenyl indolyl-3-butyramide; P-IBA, phenyl indole-3-butyrate; P-ITB, phenyl indole-3-thiolobutyrate; thiram, tetramethyl thiuramdisulfide; 2,4,5-T, 2,4,5-trichlorophenoxyacetic acid.

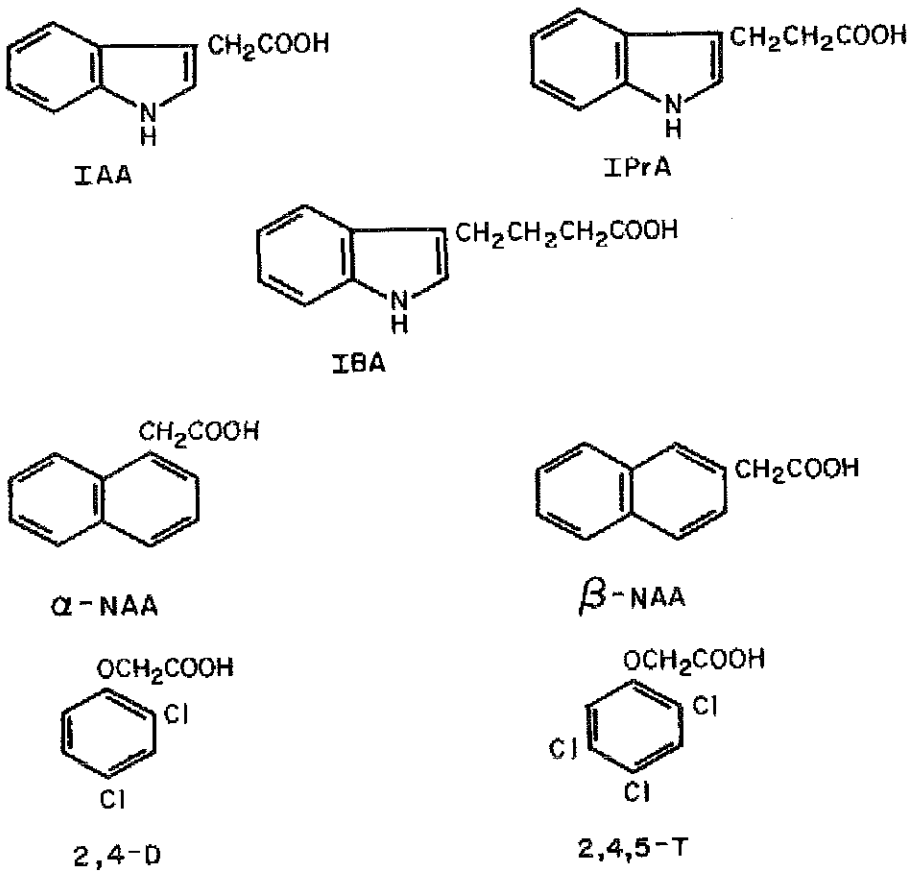
INTRODUCTION

Treatment of cuttings with various substances to promote adventitious rooting is an old concept, undoubtedly dating to the earliest attempts to vegetatively propagate plants. Although early successes were often reported, repeatability was usually impossible and positive results were limited to easy-to-root species, but even then only when proper environmental conditions were used. Luck was also an important factor in successful propagation.

In early attempts to promote rooting, some unusual practices were used, such as embedding grain seeds in the split ends of cuttings. This unusual technique used by early European and Middle Eastern propagators was later found to have a sound scientific basis because germinating grain seeds produce IAA (Hartmann and Kester 1983) (Fig. 1). Such techniques were sometimes effective but often they were unreliable and generally made rooting of cuttings more art than science. However, the unpredictable nature of rooting was removed or at least reduced in 1934–35 as a result of the chemical identification and elucidation of IAA as a rooting promoter (Thimann and Went 1934, Thimann and Koepfli 1935). These landmark papers in the history of plant propagation led to auxin[1] treatment of cuttings to promote rooting, and made it possible to consistently root large quantities of cuttings from plants that previously had been very difficult if not impossible to propagate vegetatively.

This review is a discussion of chemical compounds used to promote rooting, primarily auxins. Various topics related to auxin treatment of cuttings to stimulate rooting are considered from both practical and theoretical standpoints to encourage research directed towards improved rooting of cuttings.

Figure 1. Examples of common auxins.



[1]The term auxin(s) as used in this discussion is defined as "organic substances which at low concentrations (<0.001 mol·l^{-1}) promote growth (cell enlargement) along the longitudinal axis, when applied to shoots of plants freed as far as practical from their own inherent growth promoting substance and inhibit the elongation of roots" (Thimann 1969). Despite being involved in many physiological processes which the definition does not address, that role of auxin of greatest interest here is the initiation of adventitious roots.

CHEMICALS USED TO PROMOTE ROOTING

Following the discovery that IAA promoted adventitious rooting, the search began for other naturally occurring auxins. Also, compounds with structures similar and dissimilar to IAA were examined for root promoting properties. Searches for naturally occurring auxins were unsuccessful. Currently, it is generally agreed that IAA is the major (Moore 1979) if not the only (Thimann 1969) naturally occurring auxin found in plants, existing in both free and bound (conjugated) forms (Cohen and Bandurski 1982). Tests of IAA analogues, however, were more successful and in 1935 the first report appeared which indicated that the synthetic auxins IBA and NAA had strong root promoting properties (Zimmerman and Wilcoxon 1935) (Fig. 1). Reports of additional synthetic compounds also classified as auxins and having root promoting activity appeared in 1942 (Hitchcock and Zimmerman 1942, Zimmerman and Hitchcock 1942). These substances included the phenoxy compounds (e.g. 2,4-D; Fig. 1) which in later years would be extensively utilized as herbicides.

Since 1942, additional naturally occurring and synthetic, auxin and nonauxin compounds have been reported to stimulate rooting in cuttings (Hartmann and Kester 1983). Commercial use, however, has been limited principally to IBA and NAA, and less to IAA and phenoxy compounds such as 2,4-D and 2,4,5-T (Fig. 1). Preference for IBA and NAA is illustrated by the large number of commercial rooting formulations containing one or both of them (Table 1).

An interesting sidelight to discovery of promotion of rooting by auxins occurred in 1933, when research demonstrated that certain gases such as C_2H_2, CO, C_2H_4, and C_3H_6 promoted rooting in cuttings of various species (Zimmerman et al. 1933, Zimmerman and Hitchcock 1933). In herbaceous cuttings, the promotion of rooting by these gases was through greater root initiation and/or root development; for woody cuttings, only development of existing root primordia was greater (Zimmerman et al. 1933). Little of this knowledge has ever been used commercially. The role of C_2H_4 in adventitious rooting has continued to receive attention since an early study suggested that auxin induced production of C_2H_4 may account for the capacity of auxin to stimulate root initiation (Zimmerman and Wilcoxon 1935). However, attempts to correlate adventitious rooting with auxin mediated C_2H_4 production have been inconclusive (Mudge and Swanson 1978, Geneve and Heuser 1982). Variable results have also been reported as to the effects of applied C_2H_4-generating compounds on rooting (Krishnamoorthy 1970, Samananda et al. 1972, Mudge and Swanson 1978, Geneve and Heuser 1983, Robbins et al. 1983). Relations between auxin, C_2H_4, and adventitious rooting are complex and poorly understood (see Chapter 11 by Mudge).

Although IBA and NAA have remained for many years the principal auxins for rooting cuttings, the search continues for other compounds. In 1979, aryl esters of IAA and IBA were reported to be superior to the free acids of these same compounds in promoting root initiation (Haissig 1979). Subsequent research in 1983 demonstrated that two aryl esters (P-IBA and P-ITB, Fig. 2a) and an aryl amide (NP-IBA, Fig. 2a) were more efficient in inducing rooting of cuttings of bean (*Phaseolus vulgaris* L. cv. Top Crop) and jack pine (*Pinus banksiana* Lamb.) than IBA (Haissig 1983). Studies with other plant species have confirmed previous findings that the aryl esters of IBA may have merit in rooting cuttings (Dirr 1986, Struve and Arnold 1986). The principal benefit, however, may not be greater effectiveness than IBA but reduced toxicity (Haissig 1983, Dirr 1986). Overall, more research is needed to test the efficacy of these newly developed aryl esters and amides of indole auxins and to seek even more potent compounds.

RELATIVE EFFICACY OF AUXINS

As reports appeared indicating that treatment of cuttings with various compounds, particularly auxins, promoted rooting so did information regarding the relative efficacy of these compounds. IPrA was reported to be less effective than IAA, α-NAA more effective than β-NAA, and IBA and α-NAA more effective than IAA (Zimmerman and Wilcoxon 1935) (Fig. 1). Differences between IBA and NAA were established by rooting studies on a wide range of plant species of varying rooting ability. Although IBA and NAA had been judged to be superior to IAA, IBA was found to be more effective

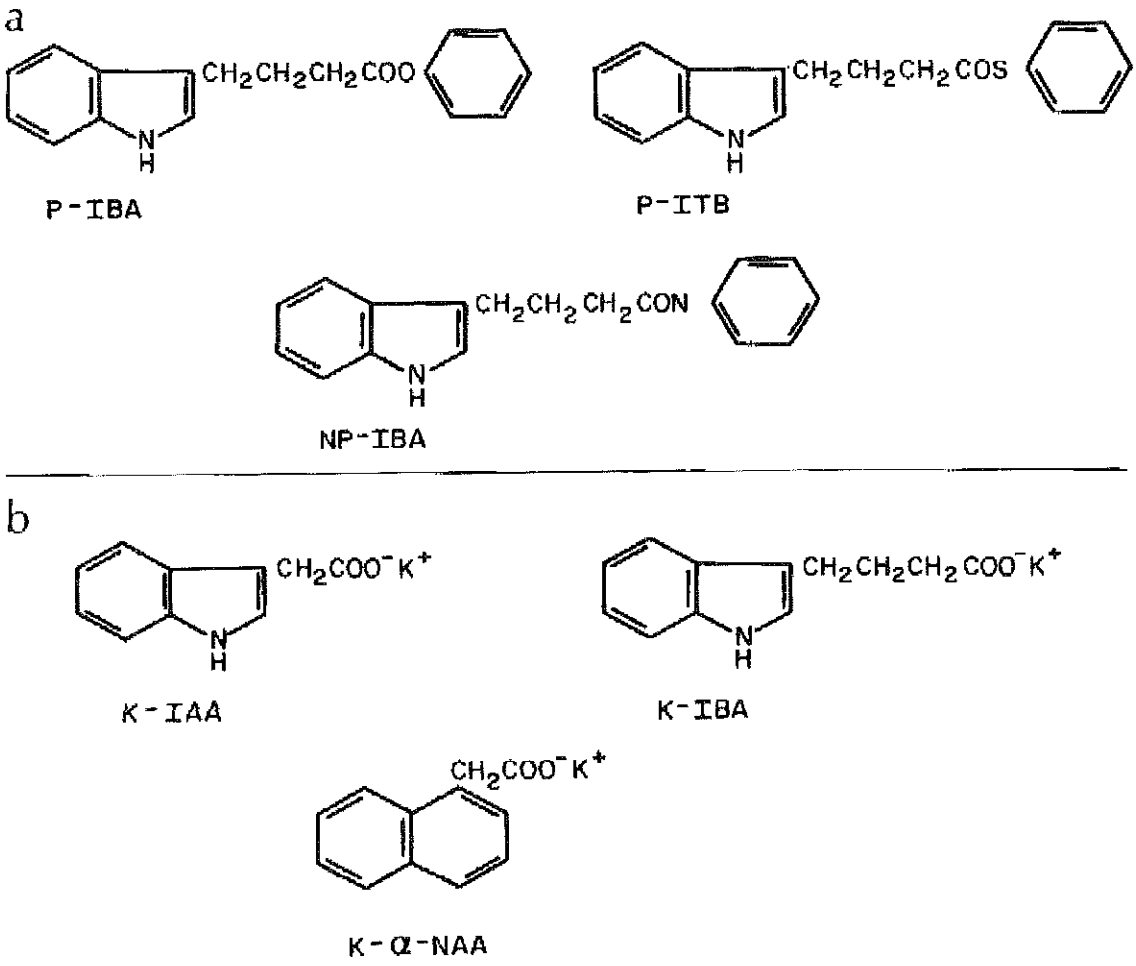


Figure 2. Aryl esters (P-IBA and P-ITB) and an aryl amide (NP-IBA) of IBA (a) and K-salts of IAA, IBA and NAA (b).

than NAA for most species (Hitchcock and Zimmerman 1939). The same report also showed that the K-salts of IAA, IBA, and NAA were consistently more effective than the corresponding acids (Fig. 2b). This knowledge has been ignored and through the years the acids have been used much more than their salts.

Although IBA was found to be more effective than NAA (Hitchcock and Zimmerman 1939), comparison of the two auxins is not simple because many factors influence auxin efficacy. For example, other factors being equal, that aspect of formulation consisting of the actual preparation in which the auxin resides (e.g. auxin-talcum powder mixture vs. an auxin solution) can influence efficacy (Hitchcock and Zimmerman 1936, 1939, Heung and McGuire 1973, Bonaminio and Blazich 1983). This aspect of formulation is not to be confused with another dealing with the chemical form of the auxin, for example, acid vs. salt. Also, a particular species may react differently when treated with equivalent concentrations ($mol \cdot l^{-1}$) of different auxins (Proebsting 1984).

Due to the considerable variation in relative auxin efficacy, studies have been conducted to determine the physiological bases for these differences. These data plus problems associated with using particular auxins explain why some auxins are used with greater frequency than others. For example, reduced root promoting activity of applied IAA, compared to IBA and NAA, has been

attributed to the fact that plant tissues possess several metabolic mechanisms that remove IAA from the growth regulating system (Leopold and Kriedemann 1975, see Chapter 9 by Gaspar and Hofinger). In simple terms, plants possess mechanisms which operate to reduce and/or nullify the effectiveness of IAA by conjugating it with other compounds or destroying it. Also, nonsterilized IAA solutions are rapidly destroyed by microorganisms (Hartmann and Kester 1983) and strong sunlight (Moore 1979, Hartmann and Kester 1983).

Phenoxy compounds, such as 2,4-D, promote rooting in cuttings of many species when used at very low concentrations. However, phenoxy compounds have not been extensively used for rooting cuttings even though they are relatively light-stable and are more resistant to microbial decomposition. There are several reasons for this, one being that following rooting these materials inhibit shoot formation, possibly because they are translocated to buds (Anon. 1983). Also, applied phenoxy compounds often stimulate callus growth on the bases of cuttings. This growth is usually associated with numerous short roots that are bent, thickened, and stubby. Masses of these short roots often appear to be fused. This aberrant form of root system markedly contrasts with the strong fibrous root systems produced after IBA treatment of cuttings. Roots produced after treatment with phenoxy compounds often develop slowly. Slow root system development reduces the overall growth rate of cuttings.

In addition, the concentration of a phenoxy compound that promotes rooting often results in necrosis of the basal portion of the cutting. If the concentration used is somewhat greater than the optimum rooting concentration, severe injury or death of the cuttings may result, which illustrates the narrow, effective range of concentrations for these compounds. The foregoing observations and experiences confirm an early report about use of phenoxy compounds to promote rooting of cuttings (Hitchcock and Zimmerman 1942).

Despite problems with using phenoxy compounds, they may be effective in combination with one or more non-phenoxy auxins. Studies have shown that if a phenoxy compound is used in combination with an auxin such as IBA, NAA, or both IBA and NAA, the rooting response is enhanced in comparison to use of a phenoxy compound alone (Hitchcock and Zimmerman 1942, Ellyard 1981). A similar improvement was obtained by using mixtues of IBA and NAA (Hitchcock and Zimmerman 1940).

The excellent root promoting properties of IBA and NAA, which were established soon after they were found to promote rooting, continue to be borne out by their widespread use for rooting cuttings. Another reason for their extensive use is that they are not easily degraded during storage. Whether initially purchased as reagent grade chemical from which particular rooting formulations are prepared, or purchased in the form of commercial rooting formulations, IBA and NAA are presently the two most widely used auxins (Table 1).

TREATING CUTTINGS WITH ROOT PROMOTING COMPOUNDS

Benefits of Treatment

Countless references can be found on the stimulatory influence of auxin treatment in rooting cuttings of difficult-to-root species. Although many such species positively respond to auxin treatment, some do not, illustrating that positive physiological response to auxin treatment is not universal. However, the benefit of auxin treatment is obvious in those species where rooting would not otherwise occur. In addition, four specific advantages associated with auxin treatment have long been recognized: 1) increasing the percentage of cuttings that form roots; 2) hastening root initiation; 3) increasing the number and quality of roots produced per cutting; and 4) increasing the uniformity of rooting (Hitchcock and Zimmerman 1936). The first, second, and fourth advantages need no explanation. However, the third regarding the number and quality of roots per cutting is intriguing because countless studies have noted it as a benefit of auxin treatment, yet published reports on the influence of root numbers on subsequent cutting growth are lacking.

Treatment Techniques

Based on reports in the 1930s that cuttings could be treated with auxins to promote rooting, various methods were tried for treatment. From this early research the main techniques for treating cuttings with root promoting compounds were developed: 1) application of auxin-talcum powder mixtures; 2) dilute-solution soaking; and 3) concentrated-solution dip (Hitchcock and Zimmerman 1939, Hartmann and Kester 1983).

Auxin-talcum powder mixtures

This method consists of dipping the basal portion (e.g. 1–2 cm) of a cutting into an auxin-talcum powder mixture. With this type of formulation, the active ingredient(s) are carried in the inert talcum powder. Auxin-talcum powder mixtures can be purchased commercially, or prepared by the user if reagent grade auxin is available. When preparing auxin-talcum powder mixtures, thorough blending of the auxin(s) and talcum powder is very important (Stoutemyer 1938, see Table 2 for procedures). Auxin-talcum powder mixtures often contain more than one auxin and may also contain a fungicide (Table 1). Treatment of cuttings with a fungicide, whether incorporated into the auxin-talcum powder mixture or used in other ways has been shown to protect newly formed roots from fungal attack, increase survival, and increase overall quality of the rooted cuttings (Doran 1952, Wells 1963, Hansen and Hartmann 1968, Fiorino et al. 1969). In addition to fungicides, other substances such as sucrose *have also been added to these preparations* (Hare 1977).

For the most effective application of auxin-talcum powder preparations, it is desirable to have some moisture at the base of the cutting so the powder will adhere (Hitchcock and Zimmerman 1939). After treatment, the cutting is lightly tapped to remove any excess powder and immediately inserted into the rooting medium. Before insertion, a trowel or similar device should be used to make a trench in the rooting medium into which the bases of the cuttings are placed. The trench should be sufficiently wide so that the powder is not rubbed off when cuttings are inserted (Stoutemyer 1938). After insertion, the rooting medium should be gently firmed around each cutting. A dibble (e.g. a wooden plant label) to make holes can also be used when inserting cuttings into the rooting medium.

When treating cuttings, it is advisable to remove a small quantity of stock material containing the auxin(s) and place it in another container that will be used for treatment. Any excess left after treatment should be discarded. Cuttings should never be dipped into the *entire stock of powder* because this may result in microbial contamination of the stock and deterioration of the auxin(s).

The major advantages of using this procedure include: 1) ready availability of commercial formulations; and 2) simplicity and ease of application. On the other hand, disadvantages are: 1) difficulty of obtaining uniform results due to potentially varying amounts of powder applied to individual cuttings; and 2) high cost of commercial powder preparations when treating extremely large quantities of cuttings. The first disadvantage also deters treating more than one cutting at a time. Thus, it is best to treat single cuttings when using auxin-talcum powder mixtures.

Dilute-solution soaking

This technique involves soaking the basal portion (e.g. 1–3 cm) of stem cuttings in a dilute solution (e.g. 20–500 $mg \cdot l^{-1}$) of one or more auxins for varying lengths of time (e.g. 2–3 up to 24 h). Following soaking, the cuttings are inserted into the rooting medium (see Table 3 for preparation of solutions).

The dilute-solution soaking method is not popular because it only has disadvantages: 1) it is slow; 2) special equipment is needed for soaking the cuttings; and 3) results can be variable because the amount of solution absorbed depends upon surrounding environmental conditions during treatment. In order to minimize (3), cuttings should be consistently treated at about 20 °C under indirect illumination (Hartmann and Kester 1983).

Concentrated-solution dip

This popular procedure is often referred to as the concentrated-dip or, simply, quick-dip. It consists of briefly (e.g. 1–5 s) dipping the basal portion (e.g. 0.5–2 cm) of a stem cutting into a concentrated (e.g. 500–30,000 $mg \cdot l^{-1}$) solution of one or more auxins, followed by insertion of the cutting

into the rooting medium. Solutions can be purchased commercially or prepared (see procedures in Table 4).

Advantages associated with this technique include: 1) greater economy if reagent grade auxin is purchased and used to prepare solutions, compared to purchasing the same amount of a commercial rooting formulation; 2) speed and ease of use; 3) uniform treatment of bundled cuttings; and 4) generally uniform results. Disadvantages are: 1) difficulty in solution preparation, particularly if non-water soluble auxin is used (i.e. acid form); 2) requires practical experience; and 3) lack of commercial preparations, compared to auxin-talcum powder formulations.

The first disadvantage concerning solution preparation warrants further explanation. The reagent grade of most auxins is synthesized as an acid. In this form, the chemical is generally insoluble in water and must be dissolved in an organic solvent such as alcohol or a strong base, e.g. aqueous NaOH. Strong bases have the disadvantage of greatly raising the pH of the solution, unless it is buffered. High pH might injure cuttings, but preparation of buffers may be inconvenient and costly. Thus, use of buffered auxin solutions has only been used in research (Haissig 1979). Ethyl, methyl, and isopropyl alcohol are all satisfactory solvents and are commonly used. It is best to avoid using full or nearly full strength (95–100%) alcohol because it may dehydrate and injure basal stem tissue. If possible, alcohol should be diluted by 50% with distilled or deionized water. However, 50% alcohol may not dissolve the very large amounts of auxin required to produce concentrated stock or treatment solutions. Also, it is often necessary to use a mechanical stirring device to completely dissolve auxins, which are only slowly soluble in alcohol. Salts of some auxins, particularly K-salts, are available commercially and should be dissolved in distilled or deionized water as opposed to tap water. Salts are usually much more expensive than acids.

Lack of practical experience with this technique can result in killing or severely injuring cuttings during treatment. Solution concentrations must be carefully adjusted to the species and degree of lignification of the cuttings; duration of treatment must also not be too long.

When using the concentrated-solution dip some precautions should be taken. For example, evaporation of alcohol can markedly elevate the concentration of auxin in solution. Excessive concentration can cause injury or death of cuttings. Dilution causing a reduction in effectiveness can also occur if the cuttings have external moisture (Blazich 1976). Concentrated (95–100%) alcohol solutions may also absorb water from humid air, resulting in dilution. To avoid changes in concentration, it is advisable to dip cuttings in only a suitably small portion of the stock solution. Any solution remaining after use should be discarded, not returned to the stock. On a large production scale, the best way to avoid an increase or decrease in concentration is to periodically use a fresh quantity of solution. Stock solutions should be kept in tightly capped bottles and stored in the dark under refrigeration.

Other methods

Although cuttings are generally treated with auxin applied either as an auxin-talcum powder mixture, or as a dilute or concentrated solution, there are other treatment methods which may have merit—e.g. methods that have been used for inducing rooting of seedlings of difficult-to-transplant species. One method has consisted of inserting toothpicks impregnated with auxin into the severed tap root and main lateral roots of recently dug plants. The toothpicks were impregnated with auxin by soaking in an auxin solution for 24 h. This procedure has proven successful in stimulating root initiation in pecan [*Carya illinoensis* Koch (Romberg and Smith 1938)], pear [*Pyrus communis* L. cv. Bartlett (Looney and McIntosh 1968)], and scarlet oak [*Quercus coccinea* Muenchh. (Struve and Moser 1984)]. Reasons for the promotive influence of this treatment may be: 1) the auxin is placed in close proximity to those cells involved in adventitious rooting; 2) a slow release effect of the auxin over time; and 3) the combination of (1) and (2) (Struve and Moser 1984).

Success with the toothpick method with seedlings suggests that it may have potential in rooting cuttings, particularly for difficult-to-root species. Unfortunately, except for one report utilizing cuttings of eastern white pine [*Pinus strobus* L. (Struve and Blazich 1982)], this technique has not been investigated. The eastern white pine study involved a comparison of application of auxin applied as a talcum powder formulation, a concentrated solution, or impregnated in toothpicks. The toothpick

method increased percent rooting and induced more roots over a longer time period than basal treatment with IBA in talc or as a concentrated solution. Inducement by the toothpick method of more adventitious roots over a longer period of time suggested that application of auxin in a slow release form may have merit in promoting adventitious rooting. Thus, gradual release of auxin to cuttings warrants further investigation.

For example, instead of using toothpicks, it may be possible to apply auxin mixed with a starch-polyacrylate gel (Starbuck and Preczewski 1986). Mixing auxin with a viscous material followed by application to cuttings is not a new concept; in one of the first techniques used, an auxin-lanolin mixture was applied to cuttings which promoted rooting (Zimmerman and Wilcoxon 1935). However, a later study utilizing cuttings of various woody species found auxin solutions to be more effective (Hitchcock and Zimmerman 1936).

Another approach for auxin application might involve use of vacuum treatment. This could possibly have merit for species and types of cuttings in which uptake of auxin solution is not easily accomplished. This was attempted but results were variable (Butterfield and McClintock 1939).

FACTORS INFLUENCING RESPONSE TO APPLIED AUXIN

Given the stimulatory response achieved by treating cuttings of various species with auxins, it is surprising how few studies have been conducted to identify factors influencing the response of cuttings to auxin application. When such investigations have been undertaken, they have been designed primarily to define how an aspect of a particular treatment technique (e.g. duration or depth of treatment associated with the concentrated-solution dip) influenced rooting response. For the purposes of this discussion, such investigations will be termed "direct studies." That term will also be used for experiments designed to examine factors also related to treatment of cuttings such as auxin efficacy and formulation. On the other hand, examination of data from other rooting experiments allows identification of additional factors influencing auxin response and these will be termed "indirect studies."

Direct Studies

One of the first factors to be studied was efficacy. As mentioned previously, an early report showed that treatments with IBA or NAA were more effective than IAA (Zimmerman and Wilcoxon 1935); IBA treatment was later found to be more effective than NAA for most species (Hitchcock and Zimmerman 1939).

Although efficacy is unquestionably important, formulation can also have a pronounced influence on rooting. There are, however, two ways to view formulation. One involves the actual chemical form of the auxin, for example, acid vs. K-salt. The second is the preparation in which the auxin resides that will be used to treat cuttings, e.g. auxin-talcum powder compared to concentrated solution.

It is difficult to discuss formulation only from a chemical standpoint. If one were comparing the acid form of IBA to the K-salt, both prepared as solutions, comparison would be confounded because the acid form is generally only soluble in an organic solvent whereas the K-salt is water soluble. Even if striking treatment differences were noted one could argue that such differences were due to the carrier, and not the chemical formulation. In any event, an early study reported that the K-salts of IAA, IBA, and NAA were more effective than the acids (Hitchcock and Zimmerman 1939).

A test to eliminate such confusion would compare the acid form of the auxin applied as an auxin-talcum powder mixture and as a concentrated solution in an organic solvent such as alcohol. Results of this type of test have generally demonstrated the superiority of the concentrated solutions over talc formulations (Heung and McGuire 1973, Bonaminio 1983, Bonaminio and Blazich 1983). A common finding of these studies has been that much less auxin is needed to obtain good rooting when concentrated solutions are used, compared to talc. Greater effectiveness of concentrated solutions has been attributed to increased uptake (Heung and McGuire 1973). Another factor which may have a bearing on the rooting response, particularly when the carrier is an organic solvent, is that solvents

such as ethanol, methanol, and acetone can stimulate rooting (Bhattacharya et al. 1985); high concentrations, however, may inhibit rooting (Middleton et al. 1978, Bhattacharya et al. 1985). Such findings also show the importance of proper solvent control treatments when conducting rooting experiments (Middleton et al. 1978).

On the other hand, talc preparations may have merit for species where slow auxin uptake at low concentrations may be beneficial (Shibaoka 1971, Heung and McGuire 1973). More experiments are needed, however, to compare effects of talcum powder and alcohol formulations on rooting.

Other experiments have defined how various aspects of the preparation and utilization of auxin formulations influence auxin response. For example, when preparing talc formulations, the manner of mixing auxin with talcum powder influences efficacy. Talc formulations of auxin prepared by initially dissolving the auxin in alcohol are superior in promoting rooting, compared to formulations prepared by initially grinding IAA crystals with talc (Heung and McGuire 1973). Other factors that influence efficacy of auxin-talc formulations include the amount of moisture at the base of a cutting, which influences adherence of the powder (Hitchcock and Zimmerman 1939); loss of powder when inserting cuttings into the rooting medium (Stoutemyer 1938); and length of the basal stem that is treated (Stoutemyer 1938).

Studies have also examined factors influencing the response of cuttings to auxin applied in dilute or concentrated solution, e.g. concentration (Nahlawi 1970); duration of treatment (Nahlawi 1970, Howard 1974); time elapsed between cutting collection and treatment (Howard 1974); length of basal stem that is treated (Nahlawi 1970, Howard 1974, Blazich 1979); position of auxin applied to the basal stem (Nahlawi 1970); wounding (Nahlawi 1970); external moisture on cuttings prior to treatment (Blazich 1978); treatment with the surface sterilant NaOCl prior to auxin treatment (Blazich 1978); and drying of solution applied to the basal stem prior to insertion of cuttings into rooting medium (Blazich 1978).

Indirect Studies

Of numerous factors influencing rooting, wounding and bottom heat have consistently been shown to modify auxin induced rooting in cuttings of many species. Wounding cuttings of particular species is known to stimulate rooting but with the exception of one previously mentioned study (Nahlawi 1970), which viewed wounding as a factor influencing auxin response, most investigators have failed to recognize wounding as a factor enhancing auxin treatment. This may have resulted because: 1) most species do not require any deliberate wounding for satisfactory rooting; or 2) stimulation by a combination of wounding plus auxin treatment for those species where wounding is not required is only slightly better than auxin treatment alone (Blazich and Bonaminio 1983). Examination of data from various rooting studies concerning the influence of wounding shows that the greatest response from wounding was generally achieved when wounding was followed by auxin treatment (Hinesley and Blazich 1981). The interaction of wounding and auxin in promoting rooting may be due to increased exposure of those cells or tissues that initiate roots as a result of auxin stimulation.

Bottom heat has also been overlooked as a factor which influences auxin response. Bottom heat involves maintaining the rooting medium at 18–24 °C. Bottom heat is generally acknowledged to stimulate rooting of cuttings, but available evidence suggests that combined bottom heat and auxin treatment better promote rooting than either treatment alone (Hinesley and Blazich 1981). Elevated temperature at the base of the cutting may enhance the rate of metabolic processes associated with rooting that are triggered by auxin. For more information on basal heating, see Chapter 18 by Loach.

STOCK PLANT TREATMENTS TO ENHANCE RESPONSE TO AUXIN

Use of particular stock plant treatments to enhance the rooting response of cuttings to applied auxin may have significance but is mostly unstudied. However, data have shown that such treatments may have an effect. For example, severe stock plant pruning may enhance auxin response

(Howard and Harrison-Murray 1985). Two other treatments that produce a positive response are girdling, and etiolation and/or blanching.

Girdling a stem results in destruction of the phloem at the point of girdling, which blocks the basipetal translocation of carbohydrates, plant hormones (e.g. auxin), and other substances that may be critical for rooting (e.g. rooting cofactors). Thus, these substances may accumulate in the stem just above the girdle and promote rooting. Girdling has promoted rooting in many species but the bases for the response are unknown. Girdling is a standard treatment when propagating various species by air layering (Hartmann and Kester 1983). Auxin treatment further improves rooting of girdled stems (Sparks and Chapman 1970), which suggests that the accumulation of root inducing factors at the point of girdling interacts additively or synergistically with auxin to promote rooting. Evidence for accumulation of root promoting substances was demonstrated in another investigation which reported that the level of a rooting cofactor increased substantially above the girdle in an easy-to-root clone of hibiscus [*Hibiscus rosa-sinensis* L. (Stoltz and Hess 1966)].

Etiolation, the total development of stock plants or their parts in the absence of light (partial development is termed blanching) has long been known to stimulate rooting (see Chapter 2 by Maynard and Bassuk). Although enhancement of rooting by these treatments is without question, factors responsible for the promotive influence are uncertain. One of the first studies aimed at elucidating the underlying physiological basis for this phenomenon concluded that increased rooting was due to a complex of interacting factors (Herman and Hess 1963). These factors included increased levels of endogenous auxin and rooting cofactors, and various anatomical changes conducive to rooting. A subsequent investigation also reported anatomical changes induced by etiolation, corresponding to increased rooting (Doud and Carlson 1977). However, another study noted that etiolation did not significantly change the auxin concentration and results suggested that etiolation stimulated rooting by increasing the sensitivity of the tissue to auxin (Kawase and Matsui 1980). It is apparent from the few studies reported that promotion of rooting by etiolation and blanching are poorly understood and need further investigation.

CONCLUSION

Auxins were discovered years ago and are still the only applied compounds that consistently enhance adventitious rooting in cuttings that have at least some natural capacity to root. In the interim there has been little improvement in the efficacy of auxins beyond that achieved with IBA, NAA, and the phenoxy compounds such as 2,4-D, which also were first tested years ago. Why, then, have new, more effective auxins not been discovered? Possibly, the apogee of auxin promoted rooting has been attained with the existing compounds, but that seems unlikely (cf. Haissig 1979, 1983). It is more likely that lack of development of root promoting auxins has resulted from general disinterest in the study of adventitious rooting, lack of a clear understanding of its economic importance, and difficulties and high cost of synthetic auxin research and development. Such research and development requires the combined skills of horticulturists, foresters, plant physiologists, organic chemists, and numerous cooperators who will conduct broad scale, practical tests and freely share their results. Based on past evidence such an approach may take years with only a small probability of success. However, the economic importance of adventitious rooting in horticulture and forestry worldwide is sufficient justification for undertaking such research, which hopefully will be done.

Additional research is also needed to determine whether there are "rooting cofactors" (auxin synergists) that, when applied alone or with auxins, markedly stimulate rooting. Evidence collected over 30 yr suggests that such compounds exist, but they have remained elusive. In part, the research cited has not been structured and implemented precisely enough to provide unequivocal evidence and, most importantly, pure, chemically characterized compounds for practical testing. Thus, future research should stress this critical area of fundamental and practical importance.

Until either more effective auxins are discovered, or the rooting cofactors are chemically characterized for practical testing, the principal improvement in efficient use of auxins will result from improvements in proprietary rooting formulations. Here too, research is needed to facilitate

auxin uptake, minimize auxin destruction, and generally encourage the health and vigor of cuttings during the critical period that precedes rooting. In such research many previously studied influences on rooting will need to be retested under modern conditions of propagation, such as fog tunnels, heated benches, media, etc. Physiological condition of stock plants must also be considered in rating proprietary rooting treatments. How stock plants are grown before cuttings are taken and how the cuttings are subsequently treated and maintained profoundly influence auxin mediated rooting responses.

Table 2. Procedures for preparing auxin-talcum powder mixtures: amounts of auxin and talc needed to prepare a rooting powder of a particular total amount, containing a specific concentration of auxin. See instructions at end of table.

Concn. (ppm)[1]	Total Weight (mg) of Auxin + Talc									
	1000	2000	3000	4000	5000	6000	7000	8000	9000	10,000
	mg auxin									
1000	1	2	3	4	5	6	7	8	9	10
1500	1.5	3	4.5	6	7.5	9	10.5	12	13.5	15
2000	2	4	6	8	10	12	14	16	18	20
2500	2.5	5	7.5	10	12.5	15	17.5	20	22.5	25
3000	3	6	9	12	15	18	21	24	27	30
3500	3.5	7	10.5	14	17.5	21	24.5	28	31.5	35
4000	4	8	12	16	20	24	28	32	36	40
4500	4.5	9	13.5	18	22.5	27	31.5	36	40.5	45
5000	5	10	15	20	25	30	35	40	45	50
5500	5.5	11	16.5	22	27.5	33	38.5	44	49.5	55
6000	6	12	18	24	30	36	42	48	54	60
6500	6.5	13	19.5	26	32.5	39	45.5	52	58.5	65
7000	7	14	21	28	35	42	49	56	63	70
7500	7.5	15	22.5	30	37.5	45	52.5	60	67.5	75
8000	8	16	24	32	40	48	56	64	72	80
8500	8.5	17	25.5	34	42.5	51	59.5	68	76.5	85
9000	9	18	27	36	45	54	63	72	81	90
9500	9.5	19	28.5	38	47.5	57	66.5	76	85.5	95
10000	10	20	30	40	50	60	70	80	90	100
12500	12.5	25	37.5	50	62.5	75	87.5	100	112.5	125
15000	15	30	45	60	75	90	105	120	135	150
17500	17.5	35	52.5	70	87.5	105	122.5	140	157.5	175
20000	20	40	60	80	100	120	140	160	180	200

Instructions for using Table 2.

1. Select from the left column the concentration (ppm) of auxin needed.
2. Select from the top of the table the total weight (mg) of the rooting preparation needed.
3. The value given where the concentration and total weight intersect is the quantity of auxin (in mg) needed.
4. The indicated quantity of auxin is adjusted to the selected total weight with talcum powder (Total weight in mg − mg auxin = mg talcum powder needed).
5. Thorough blending of the auxin and talcum powder is necessary to avoid inconsistent results and potential toxicity. The most efficient way of doing this is to dissolve the particular weight of auxin in a small quantity of 95% ethanol and then pour the solution over the prescribed weight of talcum powder. After mixing to a slurry, the mixture is dried. An excellent way to dry the mixture is use of a forced air oven at 25 °C. When preparing auxin-talcum powder mixtures use the acid formulation of the auxin.

[1]To convert parts per million (ppm) to percent, divide by 10,000.

Table 3. Procedures for preparing dilute auxin solutions: amounts of auxin and solvent needed to prepare a solution of a particular total amount, containing a specific concentration of auxin. See instructions at end of table.

Concn. (ppm)[1]	Total Volume (ml)									
	100	200	300	400	500	600	700	800	900	1000
	mg auxin									
20	2	4	6	8	10	12	14	16	18	20
40	4	8	12	16	20	24	28	32	36	40
60	6	12	18	24	30	36	42	48	54	60
80	8	16	24	32	40	48	56	64	72	80
100	10	20	30	40	50	60	70	80	90	100
120	12	24	36	48	60	72	84	96	108	120
140	14	28	42	56	70	84	98	112	126	140
160	16	32	48	64	80	96	112	128	144	160
180	18	36	54	72	90	108	126	144	162	180
200	20	40	60	80	100	120	140	160	180	200
220	22	44	66	88	110	132	154	176	198	220
240	24	48	72	96	120	144	168	192	216	240
260	26	52	78	104	130	156	182	208	234	260
280	28	56	84	112	140	168	196	224	252	280
300	30	60	90	120	150	180	210	240	270	300
320	32	64	96	128	160	192	224	256	288	320
340	34	68	102	136	170	204	238	272	306	340
360	36	72	108	144	180	216	252	288	324	360
380	38	76	114	152	190	228	266	304	342	380
400	40	80	120	160	200	240	280	320	360	400
420	42	84	126	168	210	252	294	336	378	420
440	44	88	132	176	220	264	308	352	396	440
460	46	92	138	184	230	276	322	368	414	460
480	48	96	144	192	240	288	336	384	432	480
500	50	100	150	200	250	300	350	400	450	500

Instructions for using Table 3.

1. Select from the left column the concentration (ppm) of auxin needed.
2. Select from the top of the table the volume (ml) of auxin solution needed.
3. The value given where the concentration and volume intersect is the quantity of auxin (in mg) needed.
4. The indicated quantity of auxin is dissolved in approximately 10 ml of solvent (alcohol or distilled water) and adjusted to the necessary volume with distilled water.
5. Keep solutions in a tightly capped bottle, stored in the dark, under refrigeration.

[1]To convert parts per million (ppm) to percent, divide by 10,000. Also, mg/l = ppm.

Table 4. Procedures for preparing concentrated auxin solutions: amounts of auxin and solvent needed to prepare a solution of a particular total amount, containing a specific concentration of auxin. See instructions at end of table.

Concn. (ppm)[1]	Total Volume (ml)									
	100	200	300	400	500	600	700	800	900	1000
	mg auxin									
500	50	100	150	200	250	300	350	400	450	500
1000	100	200	300	400	500	600	700	800	900	1000
1500	150	300	450	600	750	900	1050	1200	1350	1500
2000	200	400	600	800	1000	1200	1400	1600	1800	2000
2500	250	500	750	1000	1250	1500	1750	2000	2250	2500
3000	300	600	900	1200	1500	1800	2100	2400	2700	3000
3500	350	700	1050	1400	1750	2100	2450	2800	3150	3500
4000	400	800	1200	1600	2000	2400	2800	3200	3600	4000
4500	450	900	1350	1800	2250	2700	3150	3600	4050	4500
5000	500	1000	1500	2000	2500	3000	3500	4000	4500	5000
5500	550	1100	1650	2200	2750	3300	3850	4400	4950	5500
6000	600	1200	1800	2400	3000	3600	4200	4800	5400	6000
6500	650	1300	1950	2600	3250	3900	4550	5200	5850	6500
7000	700	1400	2100	2800	3500	4200	4900	5600	6300	7000
7500	750	1500	2250	3000	3750	4500	5250	6000	6750	7500
8000	800	1600	2400	3200	4000	4800	5600	6400	7200	8000
8500	850	1700	2550	3400	4250	5100	5950	6800	7650	8500
9000	900	1800	2700	3600	4500	5400	6300	7200	8100	9000
9500	950	1900	2850	3800	4750	5700	6650	7600	8550	9500
10000	1000	2000	3000	4000	5000	6000	7000	8000	9000	10000
12500	1250	2500	3750	5000	6250	7500	8750	10000	11250	12500
15000	1500	3000	4500	6000	7500	9000	10500	12000	13500	15000
17500	1750	3500	5250	7000	8750	10500	12250	14000	15750	17500
20000	2000	4000	6000	8000	10000	12000	14000	16000	18000	20000
22500	2250	4500	6750	9000	11250	13500	15750	18000	20250	22500
25000	2500	5000	7500	10000	12500	15000	17500	20000	22500	25000
27500	2750	5500	8250	11000	13750	16500	19250	22000	24750	27500
30000	3000	6000	9000	12000	15000	18000	21000	24000	27000	30000

Instructions for using Table 4.

1. Select from the left column the concentration (ppm) of auxin needed.
2. Select from the top of the table the volume (ml) of solution needed.
3. The value given where the concentration and volume intersect is the quantity of auxin (in mg) needed.
4. The indicated quantity of auxin is dissolved in a small quantity of solvent and adjusted to the necessary volume with additional solvent.
5. Keep solutions in a tightly capped bottle, stored in the dark, under refrigeration.

[1]To convert parts per million (ppm) to percent, divide by 10,000. Also, mg/l = ppm.

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Plant Hormones

Biosynthesis, Signal Transduction, Action!

Edited by

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The cover picture shows Mendel's dwarf (*le-1*, left) and tall (*LE*, right) peas. The tall, wild-type peas possess a gene encoding gibberellin 3β-hydroxylase (GA 3-oxidase) that converts GA_{20} to GA_1. GA_1 (inset) promotes stem elongation whereas GA_{20} is inactive. Tall plants possess a relatively high level of GA_1 but the level in dwarf plants is much lower. In the mutant dwarf plants the gene differs by one base and the protein by one amino acid from the wild-type tall, and the enzyme activity is $1/20^{th}$ of the level in the tall plants (see Chapters A2, B2 and B7).

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A. INTRODUCTION

A1. The Plant Hormones: Their Nature, Occurrence, and Functions

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INTRODUCTION

The Meaning of a Plant Hormone

Plant hormones are *a group of naturally occurring, organic substances which influence physiological processes at low concentrations*. The processes influenced consist mainly of growth, differentiation and development, though other processes, such as stomatal movement, may also be affected. Plant hormones[1] have also been referred to as 'phytohormones' though this term is infrequently used.

In their book *Phytohormones* Went and Thimann (10) in 1937 define a hormone as a substance which is transferred from one part of an organism to another. Its original use in plant physiology was derived from the mammalian concept of a hormone. This involves a localized site of synthesis, transport in the bloodstream to a target tissue, and the control of a physiological response in the target tissue via the concentration of the hormone. Auxin, the first-identified plant hormone, produces a growth response at a distance from its site of synthesis, and thus fits the definition of a *transported* chemical messenger. However this was before the full range of what we now consider plant hormones was known. It is now clear that plant hormones do not fulfill the requirements of a hormone in the mammalian sense. The synthesis of plant hormones may be localized (as occurs for animal hormones), but it may also occur in a wide range of tissues, or cells within tissues. While they may be transported and have their action at a distance this is not always the case. At one extreme we find the transport of

1 The following abbreviations are used throughout this book with no further definition: ABA, abscisic acid; BR, brassinosteroid; CK, cytokinin; GA gibberellin; IAA, indole-3-acetic acid

cytokinins from roots to leaves where they prevent senescence and maintain metabolic activity, while at the other extreme the production of the gas ethylene may bring about changes within the same tissue, or within the same cell, where it is synthesized. Thus, transport is not an essential property of a plant hormone.

The term 'hormone' was first used in medicine about 100 years ago for a stimulatory factor, though it has come to mean a transported chemical message. The word in fact comes from the Greek, where its meaning is '*to stimulate*' or '*to set in motion*'. Thus the origin of word itself does not require the notion of transport *per se*, and the above definition of a plant hormone is much closer to the meaning of the Greek origin of the word than is the current meaning of hormone used in the context of animal physiology.

Plant hormones[2] are a unique set of compounds, with unique metabolism and properties, that form the subject of this book. Their only universal characteristics are that they are natural compounds in plants with an ability to affect physiological processes at concentrations far below those where either nutrients or vitamins would affect these processes.

THE DISCOVERY, IDENTIFICATION AND QUANTITATION OF PLANT HORMONES.

The Development of the Plant Hormone Concept and Early Work.

The plant hormone concept probably derives from observations of morphogenic and developmental correlations by Sachs between 1880 and 1893. He suggested that "Morphological differences between plant organs are due to differences in their material composition" and postulated the existence of root-forming, flower forming and other substances that move in different directions through the plant (10).

At about the same time Darwin (3) was making his original observations on the phototropism of grass coleoptiles that led him to postulate the existence of a signal that was transported from the tip of the coleoptile to the bending regions lower down. After further characterizations by several workers of the way in which the signal was moved, Went in the Netherlands was finally able to isolate the chemical by diffusion from coleoptile tips into agar blocks, which, when replaced on the tips of decapitated coleoptiles, resulted in the stimulation of the growth of the decapitated coleoptiles, and their bending when placed asymmetrically on these tips. This thus demonstrated the existence of a growth promoting chemical that was

[2] The term "plant growth substance" is also used for plant hormones but this is a rather vague term and does not describe fully what these natural regulators do - growth is only one of the many processes influenced. The international society for the study of plant hormones is named the "International Plant Growth Substance Association" (IPGSA). While the term plant growth regulator is a little more precise this term has been mainly used by the agrichemical industry to denote synthetic plant growth regulators as distinct from endogenous growth regulators.

synthesized in the coleoptile tips, moved basipetally, and when distributed asymmetrically resulted in a bending of the coleoptile away from the side with the higher concentration. This substance was originally named *Wuchsstoff* by Went, and later this was changed to *auxin*. After some false identifications the material was finally identified as the simple compound indoleacetic acid, universally known as IAA (11).

Discovery of Other Hormones

Other lines of investigation led to the discovery of the other hormones: research in plant pathogenesis led to gibberellins (GA); efforts to culture tissues led to cytokinins (CK); the control of abscission and dormancy led to abscisic acid (ABA); and the effects of illuminating gas and smoke led to ethylene. These accounts are told in virtually every elementary plant physiology textbook, and further elaborated in either personal accounts (9, 11) or advanced treatises devoted to individual hormones (see book list at the end of the chapter) so that they need not be repeated here. More recently other compounds, namely brassinosteroids (Chapters B7 and D7), jasmonates (Chapter F1) (including tuberonic acid, Chapter E5), salicylic acid (Chapter F2), and the peptides (Chapter F3) have been added to the list of plant hormones, and these are fully covered in this book for the first time. Polyamines, which are essential compounds for all life forms and important in DNA structure, have also been categorized as plant hormones as they can modulate growth and development, though typically their levels are higher than the other plant hormones. However, as little further understanding of their exact function in plants at the cellular and molecular levels has been added in the last few years, no individual chapter has been devoted to polyamines in this edition (a chapter on polyamines can be found in the previous edition (4): 2E Chapter C1).

It is interesting to note that, of all the original established group of plant hormones, only the chemical identification of abscisic acid was made from higher plant tissue. The original identification of the others came from extracts that produced hormone-like effects in plants: auxin from urine and the fungal cultures of *Rhizopus*, gibberellins from culture filtrates of the fungus *Gibberella*, cytokinins from autoclaved herring sperm DNA, and ethylene from illuminating gas. Today we have at our disposal methods of purification (such as high performance liquid chromatography: HPLC, following solid phase extraction: SPE cartridges) and characterization (gas chromatography-mass spectrometry: GC-MS, and high performance liquid chromatography-mass spectrometry: HPLC-MS) that can operate at levels undreamed of by early investigators (Chapter G1). Thus while early purifications from plant material utilized tens or even hundreds of kilograms of tissues, modern analyses can be performed on a few milligrams of tissue, making the characterization of hormone levels in individual leaves, buds, or even from tissues within the organs much more feasible. Thus it is not surprising to see the more-recently discovered hormones being originally

identified within plant tissues. Nonetheless only brassinosteroids were identified following investigations of plant growth effects, with the discovery of jasmonates, salicylic acid and peptide hormones deriving from work on insect and disease resistance.

Immunoassay (see 2nd edition, Chapter F2) is also used for hormone quantitation, though is considered much less precise because of interfering effects of other compounds and cross reactivity. Immunoassay columns can, however, permit the very precise isolation of plant hormones prior to more rigorous physico-chemical characterization. While the exact level and location of the hormones within the individual tissues and cells is still largely elusive (Chapter G1), huge strides have been made in analyzing and localizing the expression of genes for hormone biosynthesis using sensitive techniques such as PCR (polymerase chain reaction), or the expression, in transgenic plants, of marker genes driven by promoters of one or more steps in the biosynthetic process. The location of hormone action in tissues and cells has also been investigated by examining the location of marker gene expression driven by promoters of genes known to be induced by the presence of hormone (e.g. Chapter A2).

THE NATURE, OCCURRENCE, AND EFFECTS OF THE PLANT HORMONES

Before we become involved in the various subsequent chapters covering aspects of hormone biochemistry and action it is necessary to review what hormones do. In subsequent chapters some or most of these effects will be described in more detail, whereas others will not be referred to again. It is impossible to give detailed coverage of every hormonal effect, and the reader is referred to the book list at the end of this chapter. The choice of topics for subsequent chapters has been determined largely by whether there is active research in progress in that area. Over the last few years there has been active progress in elucidating the biosynthesis, signal transduction and action of almost every hormone. Thus whereas previously the progress in understanding the action of one hormone was much better than that of another we now find increased understanding of hormone action across the board. A good case in point is cytokinin, where we now know much more about perception, signal transduction (Chapter D3) and action (Chapter C3) than just a few years ago. In fact progress on understanding one hormone as opposed to another has been leapfrogging: whereas the action of auxin at the physiological level was one of the first to be understood (Chapter C1) we still do not understand the connection between auxin signal transduction (Chapter D1) and its final action in inducing cell elongation, and while the identification of the auxin receptor was previously regarded as established, this is now regarded as far less certain. By contrast, after two decades of relatively little advance in the understanding of brassinosteroids, or even much interest in these compounds, following their discovery by extraction

from *Brassica* pollen and the demonstration of growth activity in a bean petiole bioassay, the entire biosynthetic pathway has been elucidated (Chapter B6), receptors identified (Chapter D7), mutants characterized and crosstalk with other hormones investigated (Chapter B7).

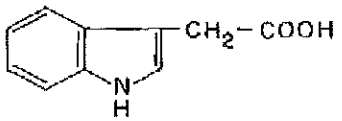
The effects produced by each hormone were initially elucidated largely from exogenous applications. However in more and more cases we have evidence that the endogenous hormone also fulfills the originally designated roles, and new functions are being discovered. Such more recent evidence derives from correlations between hormone levels and growth of defined genotypes or mutants, particularly of the model plant Arabidopsis, or from transgenic plants. In other cases it has not yet been conclusively proved that the endogenous hormone functions in the same manner.

The nature, occurrence, transport and effects of each hormone (or hormone group) are given below. (Where there is no specific chapter on the topic in this edition but a reference in the second edition of this book (4) this is indicated with the notation '2E'.) It should, however, be emphasized that hormones do not act alone but in conjunction, or in opposition, to each other such that the final condition of growth or development represents the net effect of a hormonal balance (Chapter A2) (5).

Auxin

Nature

Indole-3-acetic acid (IAA) is the main auxin in most plants.



INDOLEACETIC ACID

Compounds which serve as IAA precursors may also have auxin activity (e.g., indoleacetaldehyde). Some plants contain other compounds that display weak auxin activity (e.g., phenylacetic acid). IAA may also be present as various conjugates such as indoleacetyl aspartate (Chapter B1)). 4-chloro-IAA has also been reported in several species though it is not clear to what extent the endogenous auxin activity in plants can be accounted for by 4-Cl-IAA. Several synthetic auxins are also used in commercial applications (2E: G13).

Sites of biosynthesis

IAA is synthesized from tryptophan or indole (Chapter B1) primarily in leaf primordia and young leaves, and in developing seeds.

Transport

IAA transport is cell to cell (Chapters E1 and E2), mainly in the vascular cambium and the procambial strands, but probably also in epidermal cells (Chapter E2). Transport to the root probably also involves the phloem.

Effects

- Cell enlargement - auxin stimulates cell enlargement and stem growth (Chapter D1).
- Cell division - auxin stimulates cell division in the cambium and, in combination with cytokinin, in tissue culture (Chapter E2 and 2E: G14).
- Vascular tissue differentiation - auxin stimulates differentiation of phloem and xylem (Chapter E2).
- Root initiation - auxin stimulates root initiation on stem cuttings, and also the development of branch roots and the differentiation of roots in tissue culture (2E: G14).
- Tropistic responses - auxin mediates the tropistic (bending) response of shoots and roots to gravity and light (2E: G5 and G3).
- Apical dominance - the auxin supply from the apical bud represses the growth of lateral buds (2E: G6).
- Leaf senescence - auxin delays leaf senescence.
- Leaf and fruit abscission - auxin may inhibit or promote (via ethylene) leaf and fruit abscission depending on the timing and position of the source (2E: G2, G6 and G13).
- Fruit setting and growth - auxin induces these processes in some fruit (2E: G13)
- Assimilate partitioning - assimilate movement is enhanced towards an auxin source possibly by an effect on phloem transport (2E: G9).
- Fruit ripening - auxin delays ripening (2E: G2 & 2E:G12).
- Flowering - auxin promotes flowering in Bromeliads (2E: G8).
- Growth of flower parts - stimulated by auxin (2E: G2).
- Promotes femaleness in dioecious flowers (via ethylene) (2E: G2 & 2E: G8).

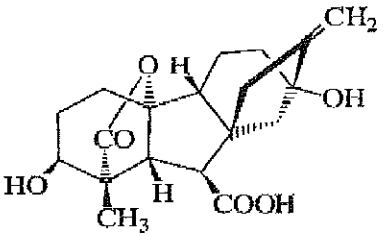
In several systems (e.g., root growth) auxin, particularly at high concentrations, is inhibitory. Almost invariably this has been shown to be mediated by auxin-produced ethylene (2, 7) (2E: G2). If the ethylene synthesis is prevented by various ethylene synthesis inhibitors, the ethylene removed by hypobaric conditions, or the action of ethylene opposed by silver salts (Ag^+), then auxin is no longer inhibitory.

Gibberellins (GAs)

Nature

The gibberellins (GAs) are a family of compounds based on the *ent*-gibberellane structure; over 125 members exist and their structures can be found on the web (Chapter B2). While the most widely available compound is GA_3 or gibberellic acid, which is a fungal product, the most important GA

in plants is GA_1, which is the GA primarily responsible for stem elongation (Chapters A2, B2, and B7). Many of the other GAs are precursors of the growth-active GA_1.



GIBBERELLIN A_1 or GA_1

Sites of biosynthesis.
GAs are synthesized from glyceraldehyde-3-phosphate, via isopentenyl diphosphate, in young tissues of the shoot and developing seed. Their biosynthesis starts in the chloroplast and subsequently involves membrane and cytoplasmic steps (Chapter B2).

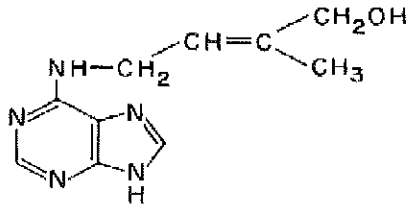
Transport
Some GAs are probably transported in the phloem and xylem. However the transport of the main bioactive polar GA_1 seems restricted (Chapters A2 and E5).

Effects
- Stem growth - GA_1 causes hyperelongation of stems by stimulating both cell division and cell elongation (Chapters A2, B7 and D2). This produces tall, as opposed to dwarf, plants.
- Bolting in long day plants - GAs cause stem elongation in response to long days (Chapter B2, 2E: G8).
- Induction of seed germination - GAs can cause seed germination in some seeds that normally require cold (stratification) or light to induce germination (Chapter B2).
- Enzyme production during germination - GA stimulates the production of numerous enzymes, notably α-amylase, in germinating cereal grains (Chapter C3).
- Fruit setting and growth - This can be induced by exogenous applications in some fruit (e.g., grapes) (2E: G13). The endogenous role is uncertain.
- Induction of maleness in dioecious flowers (2E: G8).

Cytokinins (CKs)

Nature
CKs are adenine derivatives characterized by an ability to induce cell division in tissue culture (in the presence of auxin). The most common

cytokinin base in plants is zeatin. Cytokinins also occur as ribosides and ribotides (Chapter B3).



ZEATIN

Sites of biosynthesis
CK biosynthesis is through the biochemical modification of adenine (Chapter B3). It occurs in root tips and developing seeds.

Transport
CK transport is via the xylem from roots to shoots.

Effects
- Cell division - exogenous applications of CKs induce cell division in tissue culture in the presence of auxin (Chapter C3; 2E: G14). This also occurs endogenously in crown gall tumors on plants (2E: E1). The presence of CKs in tissues with actively dividing cells (e.g., fruits, shoot tips) indicates that CKs may naturally perform this function in the plant.
- Morphogenesis - in tissue culture (2E: G14) and crown gall (2E: E1) CKs promote shoot initiation. In moss, CKs induce bud formation (2E: G1 & G6).
- Growth of lateral buds - CK applications, or the increase in CK levels in transgenic plants with genes for enhanced CK synthesis, can cause the release of lateral buds from apical dominance (2E: E2 & G6).
- Leaf expansion (6), resulting solely from cell enlargement. This is probably the mechanism by which the total leaf area is adjusted to compensate for the extent of root growth, as the amount of CKs reaching the shoot will reflect the extent of the root system. However this has not been observed in transgenic plants with genes for increased CK biosynthesis, possibly because of a common the lack of control in these systems.
- CKs delay leaf senescence (Chapter E6).
- CKs may enhance stomatal opening in some species (Chapter E3).
- Chloroplast development - the application of CK leads to an accumulation of chlorophyll and promotes the conversion of etioplasts into chloroplasts (8).

Ethylene

Nature
The gas ethylene (C_2H_4) is synthesized from methionine (Chapter B4) in many tissues in response to stress, and is the fruit ripening hormone. It does not seem to be essential for normal mature vegetative growth, as ethylene-deficient transgenic plants grow normally. However they cannot, as seedlings, penetrate the soil because they lack the stem thickening and apical hook responses to ethylene, and they are susceptible to diseases because they lack the ethylene-induced disease resistance responses. It is the only hydrocarbon with a pronounced effect on plants.

Sites of synthesis
Ethylene is synthesized by most tissues in response to stress. In particular, it is synthesized in tissues undergoing senescence or ripening (Chapters B4 and E5).

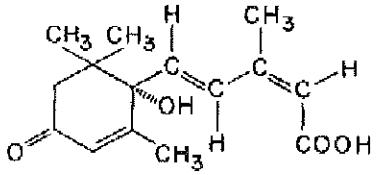
Transport
Being a gas, ethylene moves by diffusion from its site of synthesis. A crucial intermediate in its production, 1-aminocyclopropane-1-carboxylic acid (ACC) can, however, be transported and may account for ethylene effects at a distance from the causal stimulus (2E: G2).

Effects
The effects of ethylene are fully described in 2E: G2. They include:

- The so called *triple response*, when, prior to soil emergence, dark grown seedlings display a decrease in tem elongation, a thickening of the stem and a transition to lateral growth as might occur during the encounter of a stone in the soil.
- Maintenance of the apical hook in seedlings.
- Stimulation of numerous defense responses in response to injury or disease.
- Release from dormancy.
- Shoot and root growth and differentiation.
- Adventitious root formation.
- Leaf and fruit abscission.
- Flower induction in some plants (2E: G8).
- Induction of femaleness in dioecious flowers (2E: 8).
- Flower opening.
- Flower and leaf senescence.
- Fruit ripening (Chapters B4 and E5).

Abscisic acid (ABA)

Nature
Abscisic acid is a single compound with the following formula:



ABSCISIC ACID

Its name is rather unfortunate. The first name given was "abscisin *II*" because it was thought to control the abscission of cotton bolls. At almost the same time another group named it "dormin" for a purported role in bud dormancy. By a compromise the name abscisic acid was coined (1). It now appears to have little role in either abscission (which is regulated by ethylene; 2E: G2) or bud dormancy, but we are stuck with this name. As a result of the original association with abscission and dormancy, ABA has become thought of as an inhibitor. While exogenous applications can inhibit growth in the plant, ABA appears to act as much as a promoter, such as in the promotion of storage protein synthesis in seeds (Chapter E4), as an inhibitor, and a more open attitude towards its overall role in plant development is warranted. One of the main functions is the regulation of stomatal closure (Chapters D6 and E3)

Sites of synthesis
ABA is synthesized from glyceraldehyde-3-phosphate via isopentenyl diphosphate and carotenoids (Chapter B5) in roots and mature leaves, particularly in response to water stress (Chapters B5 and E3). Seeds are also rich in ABA which may be imported from the leaves or synthesized in situ (Chapter E4).

Transport
ABA is exported from roots in the xylem and from leaves in the phloem. There is some evidence that ABA may circulate to the roots in the phloem and then return to the shoots in the xylem (Chapters A2 and E4).

Effects
- Stomatal closure - water shortage brings about an increase in ABA which leads to stomatal closure (Chapters D6 and E3).
- ABA inhibits shoot growth (but has less effect on, or may promote, root growth). This may represent a response to water stress (Chapter E3; 2E: 2).
- ABA induces storage protein synthesis in seeds (Chapter E4).
- ABA counteracts the effect of gibberellin on α-amylase synthesis in germinating cereal grains (Chapter C2).
- ABA affects the induction and maintenance of some aspects of dormancy in seeds (Chapters B5 and E4). It does not, however, appear

to be the controlling factor in 'true dormancy' or 'rest,' which is dormancy that needs to be broken by low temperature or light.

- Increase in ABA in response to wounding induces gene transcription, notably for proteinase inhibitors, so it may be involved in defense against insect attack (2E: E5).

Polyamines

$$H_2N—(CH_2)_3—NH—(CH_2)_4—NH_2$$

SPERMIDINE

Polyamines are a group of aliphatic amines. The main compounds are putrescine, spermidine and spermine. They are derived from the decarboxylation of the amino acids arginine or ornithine. The conversion of the diamine putrescine to the triamine spermidine and the quaternaryamine spermine involves the decarboxylation of S-adenosylmethionine, which also is on the pathway for the biosynthesis of ethylene. As a result there are some *complex interactions between the levels and effects of ethylene and the* polyamines.

The classification of polyamines as hormones is justified on the following grounds:

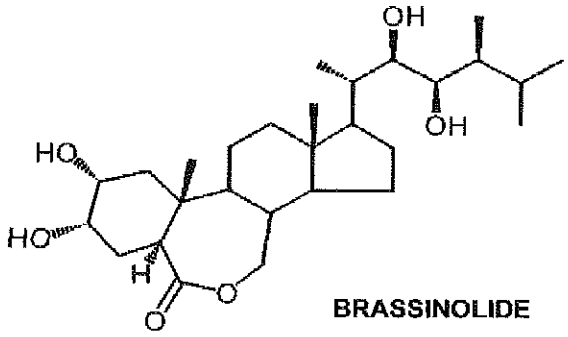
- They are widespread in all cells and can exert regulatory control over growth and development at micromolar concentrations.
- In plants where the content of polyamines is genetically altered, development is affected. (E.g., in tissue cultures of carrot or *Vigna*, when the polyamine level is low only callus growth occurs; when polyamines are high, embryoid formation occurs. In tobacco plants that are overproducers of spermidine, anthers are produced in place of ovaries.)

Such developmental control is more characteristic of hormonal compounds than nutrients such as amino acids or vitamins.

Polyamines have a wide range of effects on plants and appear to be essential for plant growth, particularly cell division and normal morphologies. At present it is not possible to make an easy, distinct list of their effects as for the other hormones. Their biosynthesis and a variety of cellular and organismal effects is discussed in 2E Chapter C1. It appears that polyamines are present in all cells rather than having a specific site of synthesis.

Brassinosteroids

Brassinosteroids (Chapters B6 and D7) are a range of over 60 steroidal compounds, typified by the compound brassinolide that was first isolated from *Brassica* pollen. At first they were regarded as somewhat of an oddity but they are probably universal in plants. They produce effects on growth and development at very low concentrations and play a role in the endogenous regulation of these processes.



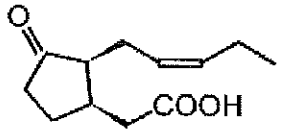
BRASSINOLIDE

Effects

- Cell Division, possibly by increasing transcription of the gene encoding cyclinD3 which regulates a step in the cell cycle (Chapter D7).
- Cell elongation, where BRs promote the transcription of genes encoding xyloglucanases and expansins and promote wall loosening (Chapter D7). This leads to stem elongation.
- Vascular differentiation (Chapter D7).
- BRs are needed for fertility: BR mutants have reduced fertility and delayed senescence probably as a consequence of the delayed fertility (Chapter D7).
- Inhibition of root growth and development
- Promotion of ethylene biosynthesis and epinasty.

Jasmonates

Jasmonates (Chapter F1) are represented by jasmonic acid (JA) and its methyl ester.



JASMONIC ACID

They are named after the jasmine plant in which the methyl ester is an important scent component. As such they have been known for some time in the perfume industry. There is also a related hydroxylated compound that has been named tuberonic acid which, with its methyl ester and glycosides, induces potato tuberization (Chapter E5). Jasmonic acid is synthesized from linolenic acid (Chapter F1), while jasmonic acid is most likely the precursor of tuberonic acid.

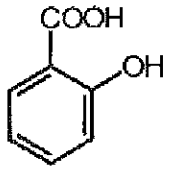
Effects

- Jasminates play an important role in plant defense, where they induce

the synthesis of proteinase inhibitors which deter insect feeding, and, in this regard, act as intermediates in the response pathway induced by the peptide systemin.

- Jamonates inhibit many plant processes such as growth and seed germination.
- They promote senescence, abscission, tuber formation, fruit ripening, pigment formation and tendril coiling.
- JA is essential for male reproductive development of Arabidopsis. The role in other species remains to be determined.

Salicylic Acid (SA)



SALICYLIC ACID

Salicylates have been known for a long time to be present in willow bark, but have only recently been recognized as potential regulatory compounds. Salicylic acid is biosynthesized from the amino acid phenylalanine.

Effects

- Salicylic acid (Chapter F2) plays a main role in the resistance to pathogens by inducing the production of 'pathogenesis-related proteins'. It is involved in the systemic acquired resistance response (SAR) in which a pathogenic attack on older leaves causes the development of resistance in younger leaves, though whether SA is the transmitted signal is debatable.
- SA is the calorigenic substance that causes thermogenesis in *Arum* flowers.
- It has also been reported to enhance flower longevity, inhibit ethylene biosynthesis and seed germination, block the wound response, and reverse the effects of ABA.

Signal Peptides

The discovery that small peptides could have regulatory properties in plants started with the discovery of systemin, an 18 amino acid peptide that travels in the phloem from leaves under herbivore insect attack to increase the content of jasmonic acid and proteinase inhibitors in distant leaves, so protecting them from attack (Chapters F1 and F3). Since then, over a dozen peptide hormones that regulate various processes involved in defense, cell

division, growth and development and reproduction have been isolated from plants, or identified by genetic approaches (Chapter F3). Among these effects caused by specific peptides are:

- The activation of defense responses.
- The promotion of cell proliferation of suspension cultured plant cells.
- The determination of cell fate during development of the shoot apical meristem
- The modulation of root growth and leaf patterning in the presence of auxin and cytokinin
- Peptide signals for self-incompatability.
- Nodule formation in response to bacterial signals involved in nodulation in legumes.

Are the More-Recently-Discovered Compounds Plant Hormones?

Two decades ago there was a heated discussion as to whether a compound had to be transported to be a plant hormone, and could ethylene therefore be a plant hormone. To this Carl Price responded: "Whether or not we regard ethylene as a plant hormone is unimportant; bananas do..."[3]. Hormones are a human classification and organisms care naught for human classifications. Natural chemical compounds affect growth and development in various ways, or they do not do so. Clearly brassinosteroids fit the definition of a plant hormone, and likely polyamines, jasmonates salicylic acid and signal peptides also can be so classified. Whether other compounds should be regarded as plant hormones in the future will depend on whether, in the long run, these compounds are shown to be endogenous regulators of growth and development in plants in general.

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B. HORMONE BIOSYNTHESIS, METABOLISM AND ITS REGULATION

B1. Auxin Biosynthesis and Metabolism

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INTRODUCTION

Auxins function at the intersection between environmental and developmental cues and the response pathways that they trigger (Fig. 1).

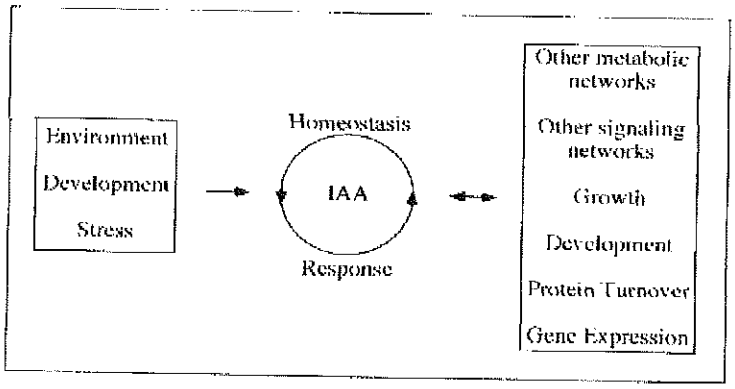

Fig. 1. Auxin homeostasis, the maintenance of an IAA level in the plant cell that is optimal for growth and development, is dependent on abiotic and biotic sources that impinge on the system, and results in a variety of often interconnected responses that themselves impinge on the system. Some of the intricate regulatory networks have evolved with considerable redundancy and adaptive plasticity to maintain optimal auxin levels in response to changing environmental and developmental conditions.

Auxin levels vary dramatically throughout the body and life of the plant, forming gradients that are a central component of its action (4, 5, 14, 20, 34). Accordingly, plants have evolved intricate regulatory networks with considerable redundancy and adaptive plasticity to maintain auxin levels in response to changing environmental and developmental conditions. We refer to this phenomenon as auxin homeostasis; specifically the biosynthesis, inactivation, transport, and inter-conversion pathways that regulate and maintain auxin levels.

The study of auxin homeostasis is aimed at understanding the mechanisms by which plants manage to have the hormone available in the required amount at the right time and place, and at determining how the developmental and environmental signals impact these processes. A combination of molecular genetic and analytical approaches in the past ten years has resulted in an increased understanding of auxin homeostasis; primarily that it involves a highly interactive network of redundant pathways, the complexity of which are only beginning to be discerned. In this chapter, we will focus our attention on the naturally occurring auxins that have been described to date: IAA, IBA[1], PAA and 4-Cl-IAA (Fig. 2). We will summarize the growing body of knowledge concerning the enzymes and the genes involved in IAA synthesis and metabolism. Several other reviews provide a more detailed analysis of particular aspects of auxin synthesis and metabolism (3, 7, 12, 13, 29, 31, 40, 56).

Chemical Forms of Auxins in Plants

Auxins are defined as organic substances that promote cell elongation growth when applied in low concentrations to plant tissue segments in a bioassay. In addition to the most often studied auxin, IAA, there are several other native auxins that have been reported to occur in plants. All natural auxins are found in plants as the free acid and in conjugated forms (Fig. 2).

The halogenated derivative 4-Cl-IAA has been found in peas, several other members of the Fabaceae and in seeds of pine (2). In many biological tests, 4-Cl-IAA is more active than IAA; for example, it has approximately 10 times the activity of IAA on oat coleoptile elongation (38). Most of the 4-Cl-IAA occurs as the methyl ester in many of the plants examined, however, 4-Cl-IAAsp and its monomethyl ester have also been described (2). Studies into the physiological role of 4-Cl-IAA have focused on its activity in pea pod development (44). Normal pod development requires seeds, but in their absence, 4-Cl-IAA and GAs act synergistically to promote pea pod development, specifically cell elongation and division (44). Expression of PsGA3ox1, the gene encoding the oxidase that converts GA_{20} to biologically active GA_1 is developmentally and spatially regulated during pod

[1] Abbreviations: Asp, aspartate; Gln, glutamine; Glu, glutamate; Gluc, glucose; IAOx, indole-3-acetaldoxime; IBA, indole-3-butyric acid; IG, indole methyl glucosinolate; Inos, inositol; Lys, lysine; OxIAA, 2-oxindole-3-acetic acid; PAA, phenylacetic acid; Trm, *tryptamine; Trp, tryptophan; Trp-D, tryptophan dependent; Trp-I, tryptophan independent.*

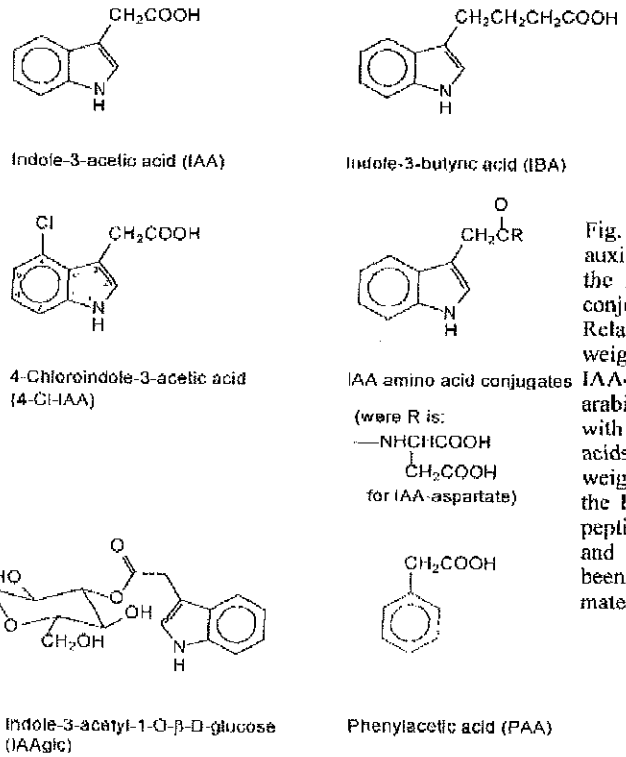

Fig. 2. Naturally occurring auxins and some examples of the lower molecular weight conjugates found in plants. Related low molecular weight con-jugates (such as IAA-Inos, IAA-Inos-arabinose, and conjugates with several other amino acids) and higher molecular weight conjugates (such as the IAA protein IAP1, IAA-peptides, IAA glycoprotein, and IAA-glucans) have also been isolated from plant materials.

development (44). Additionally, it is dramatically up-regulated by exogenous 4-Cl-IAA suggesting that 4-Cl-IAA is necessary for GA synthesis during pea pod development. PsGA3ox1 expression is not up-regulated by IAA and IAA has very little effect on stimulating GA synthesis or pod development (36). However, IAA clearly affects expression of GA biosynthesis genes in pea shoots and in tobacco (52), although the primary targets of IAA regulation are different in the two species.

IAA and its proposed precursors undergo metabolic conversions to indole-3-lactic acid, indole-3-ethanol and IBA (10). IBA has been used commercially for plant propagation for decades because of its efficacy in the stimulation of adventitious roots (2). The observation that IBA is more efficient than IAA at inducing rooting may be partially explained by the observation that IBA is more stable than IAA against *in vivo* catabolism and inactivation by conjugation (3). IBA was definitively shown to occur naturally in plants in 1989 (31). Feeding studies and analysis of metabolic mutants show that plants are able to convert IBA to IAA by a mechanism that parallels fatty acid β-oxidation (3) and to convert IAA to IBA (31). It is still not clear whether IBA is an auxin *per se*, or a precursor to IAA. Arabidopsis mutants that were resistant to the inhibitory effects of exogenous IBA on root elongation revealed a number of loci that are involved in β-

oxidation of fatty acids in the peroxisome, peroxisome protein import and possibly IBA signaling and response (3). The functions of these corresponding genes strongly suggest that IBA metabolism is centered in the peroxisome (3).

In addition to the indolic auxins, various phenolic acids in plants (such as PAA) have low auxin activity. In *Tropaeolum majus* (nasturtium), PAA is present at levels 10- to 100-fold lower than those of both IAA and IBA and it appears to be distinctly localized (32). The low biological activity, the low levels found in some plants, and its unique distribution within the plant, suggest that PAA either does not act *in planta* as an auxin or has an as yet unknown specialized function.

In most tissues, the majority of the auxin is found conjugated to a variety of sugars, sugar alcohols, amino acids and proteins (Fig. 2) (2, 56). Enzyme activities for synthesis and hydrolysis of various conjugated forms are known to exist, suggesting that these forms function to provide a readily accessible and easily regulated source of free IAA without *de novo* synthesis. One general class of conjugated forms consists of those linked through carbon-oxygen-carbon bridges. These compounds have been referred to generically as "ester-linked", although some 1-*O* sugar conjugates such as 1-*O*-IAA-Glue are actually linked by acyl alkyl acetal bonds. Typical ester-linked moieties include 6-*O*-IAGlue, IAA-Inos, IAA-glycoproteins, IAA-glucans and simple methyl and ethyl esters. The other type of conjugates present in plants are linked through carbon-nitrogen-carbon amide bonds (referred to as "amide-linked"), as in the IAA-amino acid and protein and peptide conjugates (see Fig. 2). IAA conjugates have been identified in all plant species examined for them (56). The conjugate pool in endosperm tissues of monocots and dicots is generally comprised of ester-linked moieties, whereas conjugate moieties in most dicot seeds seem to be predominantly amide-linked. Light grown vegetative tissues of most plants, both monocots and dicots, appear to contain primarily amide-linked conjugates.

Historically, our knowledge of IAA conjugate function comes mainly from tissue culture and bioassays. Various conjugates of IAA, both ester- and amide-linked, act as "slow release" forms of IAA in tissue culture (2) and when used to stimulate rooting of cuttings. Early studies showed that the biological effect of the conjugate was related to its ability to release IAA when applied to tissue (2). Differential growth induced by IAA-conjugates applied to stems of bean (*Phaseolus vulgaris*) was quantitatively correlated to the degree of hydrolysis of the conjugate by the tissue (56). Conjugated auxins are thought to be either storage or long distance mobile forms of the active hormone, or, in the case of IAA-Asp, to act as an intermediate in the degradation of IAA. There are, however, some indications that conjugates themselves can have specialized auxin activities (1, 3, 43, 60, 63).

Structural determinants of IAA function

The search for a simple structure-activity correlation model valid for IAA and a series of alkylated and halogenated derivatives has not defined a simple or straightforward relationship, although detailed analysis of their structural parameters and their physico-chemical properties has evolved our concepts of activity/structural determinants (37). Contrary to expectations, a simple relationship based on the relative lipophilicity of the compounds does not account for differences in biological activity nor does weak π-complexing ability or reduced indole ring (NH) acidity. These factors had been suggested to explain the differences in auxin activity. The ability of modified structures to function as active auxins may be defined by the relationship between the acidic side chain and unspecified properties of the ring system (37).

IAA HOMEOSTASIS PATHWAYS

Biochemical pathways that result in IAA production within a plant tissue are illustrated in Fig. 3. They include: (A) *de novo* synthesis, whether from tryptophan [referred to as Trp-dependent (Trp-D) IAA synthesis], or from indolic precursors of Trp [referred to as Trp-independent (Trp-I) IAA synthesis, since these pathways bypass Trp]; (B) hydrolysis of both amide- and ester-linked IAA conjugates; (C) transport from one site in the plant to another site; and (D) conversion of IBA to IAA. IAA turnover mechanisms include: (E) oxidative catabolism; (F) conjugate synthesis; (G) transport away from a given site; and (H) conversion of IAA to IBA. This model for inputs and outputs to the IAA pool has remained essentially the same for the past twenty years (11).

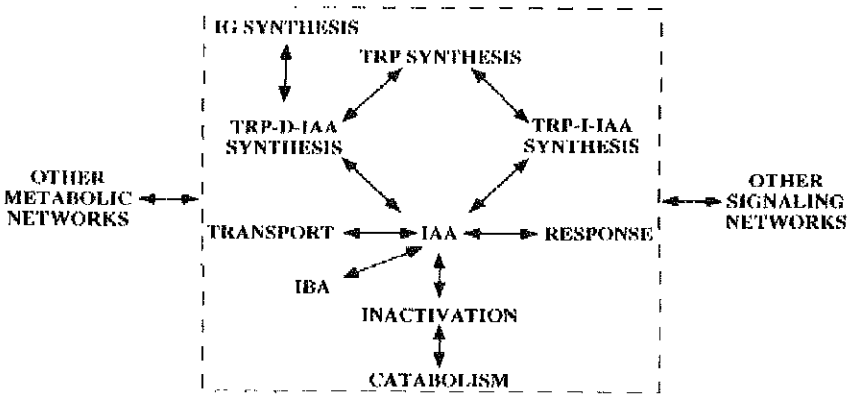

Figure 3. A diagram of our current understanding of metabolic and signaling interactions shows the major interactions discussed. There still remain a number of gaps in our understanding of the complete pathways and broader interactions between auxin regulation and other signaling networks, but hypotheses generated from the interactions shown should serve as a guide to future research efforts.

Classical genetic approaches, such as screening for auxin-deficient mutants, have yielded little information about IAA homeostasis. This has been attributed to the likelihood that auxin auxotrophs would be lethal and that IAA homeostatic pathways are redundant. Thus, a mutation in one pathway would be compensated by another functional pathway. Fortunately, the manner in which IAA metabolism is now studied has yielded many more details about the pathways and genes involved. Early characterization of IAA synthesis and metabolism involved the use of radiolabeled precursors (usually Trp) applied to cut surfaces or extracts of plants. Radiolabeled intermediates were then identified, IAA and IAA metabolites quantified, and the enzymes that catalyze these reactions isolated and characterized. This type of approach has been widely useful in delineating many metabolic pathways, with the caveat that only the capacity of the plant to carry out the metabolic reaction has been demonstrated, not the physiological relevance of the reaction. Recent advances in mass spectrometry have allowed researchers to replace radiolabeled tracer compounds with stable isotope labeled precursors that are monitored by mass spectrometry. Thus, the endogenous, unlabeled compounds of interest could be monitored as well (39). Most of these experiments are done with intact plants, providing a better biological context than previous experiments with extracts or cut sections of plants. Mutants and transgenic plants with altered IAA homeostasis have been integral to these studies, as they reveal the compensatory mechanisms that plants utilize to achieve IAA homeostasis (Fig. 4). Several major findings have emerged (29). They are that: 1) plants utilize multiple pathways for the homeostasis of IAA; 2) these pathways are differentially regulated; and 3) numerous environmental factors including light, temperature and biotic or abiotic stress regulate the activity of these pathways.

De novo synthesis

De novo synthesis refers to the synthesis of the heterocyclic indole ring from non-aromatic precursors. The only known source of indole ring compounds found in nature is the shikimic acid pathway, which is exclusive to bacteria, fungi and plants (Fig. 5). One of these compounds, the abundant amino acid Trp, contains all the carbon and structural features necessary to make IAA.

IAA Synthesis from Tryptophan

Trp-D-IAA synthesis is induced by wounding, temperature and developmental cues

An enormous body of knowledge demonstrates that IAA can be synthesized from Trp, and a summary of some of the known reactions is presented in Fig. 5. These pathways were largely elucidated by radiotracer labeling studies and biochemical assays. While they were proposed decades ago, there have always been some significant aspects of these pathways to regard with caution (42). The pool size of Trp is three or more orders of magnitude

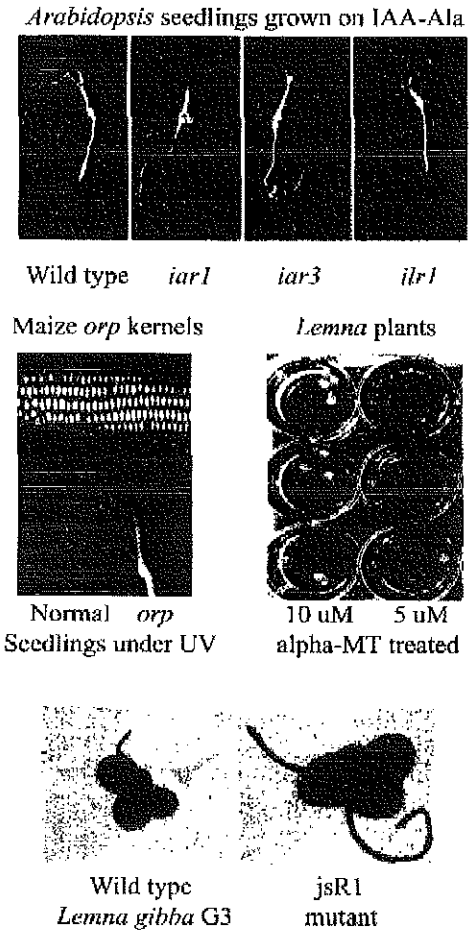

Fig. 4 (Color plate page CP2). Tryptophan or auxin metabolism mutants have served as important tools for understanding IAA metabolism. This figure illustrates some of the mutants that have enhanced our knowledge of IAA conjugate hydrolysis, the redundancy of IAA biosynthesis, and the complexity of IAA homeostasis. Shown are: [Top row] Arabidopsis mutants resistant to high levels of IAA-alanine were selected by failure of the conjugate to suppress root growth. These mutants identified genes involved in IAA conjugate hydrolysis (3). [Middle row, left] Kernels of maize *orange pericarp* (*orp*) segregate 15:1 as expected for a recessive trait encoded the two genes for tryptophan synthase β in maize. Seedlings from *orp* fluoresce brightly under UV illumination due to the accumulation of the glucoside of anthranilic acid, while normal seedlings do not. The *orp* mutant demonstrated the existence of a Trp-I pathway for IAA biosynthesis (56). [Middle row, right] Selection of lines of *Lemna gibba* resistant to α-methyltryptophan yielded plants that produced elevated levels of tryptophan due to loss of feedback inhibition of anthranilate synthase. These lines were used to show that the rate of IAA production/turnover could change without altering the steady state levels of IAA (29). [Bottom row] A large variant, jsR_1, of *Lemna gibba* was shown to have high levels of free IAA, and provided the first quantitative data indicating that a Trp-I pathway to IAA was active in plants (56).

larger than that of IAA. Trp is readily converted to IAA by microorganisms and even a minute amount of microbial conversion of Trp would produce the picomolar amounts of IAA commonly found in plants. Radiolabeled Trp can be non-enzymatically converted to IAA, presumably by decomposition mediated by peroxides and free radicals that accumulate in the radioactive solution. In addition, many of the early studies demonstrating Trp-D-IAA synthesis employed cut sections (39) and we now know that wounding induces Trp-D-IAA synthesis (61). Therefore, many of the earlier studies with tissue sections probably overestimated the contribution of Trp-D-IAA synthesis. Stable isotope labeling studies performed with intact plants have confirmed the existence and utilization of Trp-D-IAA synthetic pathways in a variety of species (29, 56). Trp-D-IAA synthesis is also induced by environmental factors such as light and temperature (49). While a direct correlation to Trp-D-IAA synthesis has yet to be made, there are several

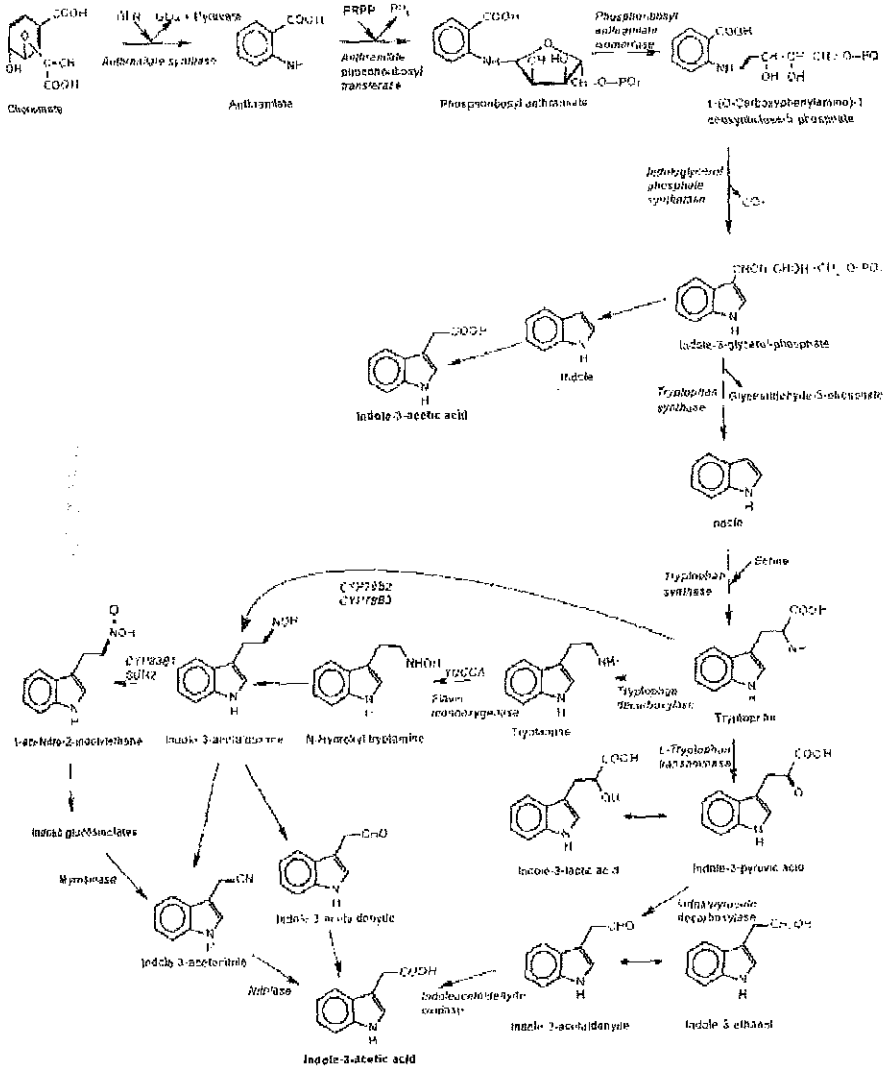

Figure 5. Summary of the reactions leading from chorismate (the first committed step of indolic metabolism) to IAA and tryptophan. In maize, the production of free indole, independent of tryptophan synthase β, has been shown to by catalyzed by two genes, *BX1* and *IGL* (17). In addition to YUCCA, the gene FLOOZY from petunia has been shown to encode a similar flavin monooxygenase (12, 64). As noted in the text, indolic glucosinolates are present only in a limited number of plant species and thus reactions involving these compounds are likely different in other plants. In fact, most of what we know on this scheme is derived from studies of only a small number of plant species.

examples of surges in auxin levels that correspond with developmental or environmental stimuli. Such surges follow zygotic fertilization (51) and changes in temperature (29). IAA metabolism is also perturbed by pathogen infection (40).

Identification of genes involved in Trp-D-IAA synthesis

Most of the Trp-D-IAA synthesis pathways shown in Fig. 5 were postulated as many as 55 years ago, yet identification of genes that encode enzymes responsible for some of the reactions has only come about within the past decade. (see Fig. 5). Genes encoding nitrilases that are able to convert IAN to IAA *in vitro* were isolated in a screen that was completely unrelated to IAA biosynthesis, and their identification was based on homology to microbial nitrilases (3). There are 4 nitrilase genes in Arabidopsis with varying degrees of affinity for IAN as a substrate (3, 48). Demonstrating a precise role for nitrilases in IAA synthesis is complicated by the redundancy of the genes. The *nit1* mutant of Arabidopsis is resistant to high levels of exogenous IAN, because the NIT1 protein is not available to convert IAN to IAA (41). However, IAA levels are normal in this mutant. The challenge in using genetic approaches to assign function to a member of a gene family is to discover those conditions under which gene redundancy does not provide adequate compensation for a defect in one member of a gene family. For example, Arabidopsis NIT3 may in fact be specifically involved in IAA synthesis in cases of sulfur starvation, as this nitrilase gene is the only one that is up-regulated under such conditions (27). In another case, nitrilase activity is up-regulated during pathogen attack, specifically by infection of Arabidopsis by *Plasmodiophora brassicae*, the causal agent of clubroot disease (40, 41). It remains to be seen just how ubiquitous the nitrilase pathway is, as IGs, which are presumed to be the likely precursors to IAN, are limited in the plant kingdom to the order Capparales (54). Tobacco and maize both encode nitrilases, but IAN levels are not believed to be significant in these species.

Genes have been identified for steps in the metabolism of Trp to IAOx (7), which in Arabidopsis is a branch point compound between IAA and IG synthesis pathways (12, 69, see Fig. 5). Several of the genes involved in Trp metabolism to IAA or IG encode enzymes that belong to the cytochrome P450 family of monooxygenases, a large group of heme-containing enzymes that primarily catalyze hydroxylation reactions. P450 enzymes are involved in biosynthesis of a number of plant hormones (26) and secondary metabolites such as the glucosinolates, which are a class of amino acid-derived plant defense compounds with anti-cancer properties (67).

Mutant analysis has revealed cross talk between IAA synthesis and IG synthesis. Specifically, altered flux through the IG pathway perturbs IAA levels (12), which is not surprising given that the two pathways share a common intermediate. What is surprising it that the biosynthetic pathways for glucosinolates derived from amino acids other than Trp can also feed into IAA homeostasis pathways in a more global regulatory network (8, 12, 21, 50). For example, *CYP79F1* is involved in synthesis of short-chain methionine-derived aliphatic glucosinolates. The bushy phenotype of the *CYP79F1* null mutant *(bus1-1f)* is indicative of a perturbation in IAA homeostasis and indeed IAA, IAN and IG levels are altered in this mutant (50). Mutations in *CYP83B1*, which directs IAOx toward IG synthesis, result

in elevated IAA and IAN levels (12) as well as increased transcription of Trp biosynthetic genes and a myb transcription factor gene, ATR1 (57). The physiological role of this transcription factor has yet to be determined; however the corresponding increase in Trp synthetic genes in CYP83B1 mutants could be explained by up-regulation of a positive regulatory factor. Ethylene, jasmonic acid and salicylic acid, elicitors of defense signaling pathways, stimulate increased CYP79B2 and CYP79B3 expression along with IG accumulation (6, 33), while non-indole glucosinolates were not affected. IAA levels have not been measured under conditions of elicitation, so the degree to which IAOx is directed between IG and IAA synthesis pathways in a defense response is not yet known.

At least one of the flavin monooxygenase-like YUCCA proteins of Arabidopsis is able to convert Trm to N-hydroxyl Trm *in vitro*, and overexpression of three of the nine related YUCCA genes in Arabidopsis results in over-production of IAA *in planta* (12, 68). Thus, members of this gene family are implicated in Trp-D-IAA synthesis, and by a novel pathway that had not been postulated from earlier biochemical approaches (Fig. 5). In a screen of activation tagged Arabidopsis mutants, the YUCCA mutants stood out as having phenotypes consistent with altered IAA levels (68). Knockouts of individual YUCCA genes were not affected in IAA homeostasis, which is not surprising given the redundancy of this gene family and of IAA synthesis pathways in general. Conventional loss of function genetic approaches are not likely to reveal redundant pathways, while overexpression approaches apparently do. The *FZY* gene of petunia appears to be an ortholog of the YUCCA family, and the *fzy* mutant exhibits blockage in the early stages of floral organ primordia formation and aberrant leaf vein patterning (12, 64). While *FZY* appears to be a single gene in petunia, auxin levels are normal in the *fzy* mutant, indicating that if these genes normally play a role in IAA synthesis, there are alternate pathways available.

De Novo Synthesis of IAA Not Involving Tryptophan

Trp-I-IAA synthesis

Since the first studies in the 1940s showing that plants have the capacity to convert exogenous Trp to IAA, many have equated IAA synthesis from Trp with *de novo* biosynthesis. Although doubts about this general concept have been raised since the early 1950s (29), only more recently have the methods for exacting studies on this question been developed. Two advances have been critical to the demonstration that plants have one or more Trp-I-IAA synthesis routes; the use of stable isotope tracer methods with mass spectrometric analysis and the availability of Trp auxotrophic mutants of higher plants (Fig 4). These methods have also resulted in critical reevaluations of when in a plant's life IAA is made *de novo*, and these studies have yielded surprising results.

The measurement of *de novo* synthesis of aromatic ring compounds can

be followed by allowing plants to grow in the presence of water enriched in deuterium oxide. Under these conditions, any newly formed aromatic rings have deuterium locked into non-exchangeable positions on the ring. Such labeling techniques provide at least two advantages. First, since the "labeled precursor" is water, such an approach does not require exact knowledge of precursors or pathways in order to accurately ascertain the extent of *de novo* synthesis. Second, since all cell compartments are freely permeable to water, problems of compartmentation and uptake are not an issue. Experiments from several laboratories in which young plants of *Zea mays*, Arabidopsis, or pea, and cell cultures of carrot were grown on 30% deuterium oxide clearly showed that IAA is made in such a way that deuterium is incorporated into non-exchangeable positions of the indole ring of IAA to a greater extent than that found in the indole ring of Trp. These results indicated that Trp is not the only precursor to IAA (2, 42).

A more exacting procedure for isotopic labeling is possible by using specific labeling with stable isotopes of carbon or nitrogen. This allows quantitative evaluations of the rates of metabolism and precise analysis of both the labeled and unlabeled compounds (2, 42). Perhaps the most striking of these isotopic labeling studies used the *orange pericarp* (*orp*) mutant of maize (2, 42 56, see Fig. 4). *Orp* carries a double recessive trait caused by mutations in the two genes encoding the enzyme for Trp synthase β. Despite this metabolic block in the terminal step for Trp biosynthesis, mutant seedlings produced IAA *de novo* and, in fact, accumulated up to 50 times the level of IAA as do non-mutant seedlings. Labeling studies established that the *orp* mutants were able to convert [^{15}N]anthranilate to [^{15}N]IAA but did not convert [^{15}N]anthranilate to [^{15}N]Trp. Neither *orp* seedlings nor control seedlings converted [$^{2}H_5$]Trp to [$^{2}H_5$]IAA in significant amounts even when the orp seedling were fed levels of [$^{2}H_5$]Trp high enough to reverse the lethal effects of the mutation (2, 42, 56). These results were the first to clearly establish that Trp-I-IAA biosynthesis does occur, and they suggested that in these plants, Trp-I-IAA biosynthesis actually predominates over the Trp-D-IAA biosynthesis pathway.

Despite the demonstration that IAA biosynthesis can occur without the amino acid Trp as a precursor, the exact pathway for Trp-I-IAA biosynthesis remains elusive. *In vivo* labeling techniques using Arabidopsis mutants confirmed and extended the *orp* findings, and suggested that the branch point for IAA production occurs at indole or its precursor, indole-3-glycerol phosphate (2, 40, 42). The pathway has also been directly demonstrated in an *in vitro* system using enzyme(s) from light grown seedlings of normal or *orp* maize. This system catalyzed the direct conversion of [^{14}C]indole, but not [^{14}C]Trp, to [^{14}C]IAA, and the addition of unlabeled Trp to the reaction mixture did not alter the rate of conversion of [^{14}C]indole to [^{14}C]IAA (3, 29, 40).

IAA is not the only compound made by plants from precursors that can lead directly to tryptophan. Camalexin, an Arabidopsis defense compound, appears to be produced from indole directly and not through Trp (70). In

maize, which does not produce camalexin, a remodeling of indolic metabolism occurs as part of defense responses and two genes, *bx1* and *igl*, produce free indole independent of Trp biosynthesis. The first gene yields the chemical protectant DIMBOA and the second gene responds to a herbivore elicitor (17).

Evidence showing the existence of a Trp-I-IAA biosynthetic pathway must be viewed in the context of compelling evidence that many plant species have been shown to convert Trp to IAA and in some cases at rates that make it important for the auxin economy of the plant. With this in mind, some of the strongest evidence supporting the importance of Trp-I-IAA biosynthesis comes from studies where, in the same plant and/or tissue, a switch occurs from one biosynthetic pathway to the other. In embryogenic carrot suspension cultures, the conversion of Trp to IAA is the primary route (29). However, following induction of these cells to form somatic embryos, the Trp-D-IAA pathway was diminished and the Trp-I-IAA pathway predominated. Similarly, IAA production in expanding green fruit of tomato occurred via Trp-D-IAA routes, but continued IAA production in red-ripe fruit was predominantly by the Trp-I-IAA biosynthesis pathway (16).

The change from one pathway to the other is not only controlled by developmental programs, but is also impacted by external signals. To study these changes, specialized techniques capable of measuring metabolic events over short time periods were required. Early experiments using stable isotope labeling over several days following the surgical removal of the cotyledons showed that in germinating axes of bean seedlings, Trp-D-IAA biosynthesis accounted for essentially all of the IAA production. When reexamined in a study using rapid labeling techniques (61) Trp-D-IAA synthesis was found to be operative only in the first hours after cotyledon removal, followed by several days when the Trp-I-IAA biosynthesis pathway predominated. Clearly then, bean uses both the Trp-I-IAA pathway and the Trp-D-IAA routes, and in this case Trp-D-IAA synthesis is activated as a consequence of the wounding resulting from cotyledon removal.

Growth temperature also impacts regulation of the IAA synthesis pathways (49) and also changes the amount of IAA that accumulates in the plant (29). In *Lemna*, low temperature growth ($\leq$ 15°C) resulted in a measured increase in IAA levels, greatly reduced the rate of growth, and initiated the early events leading to the development of resting structures known as turions. Growth at 30°C resulted in normal growth rate and IAA levels (49). When *Lemna gibba* was grown at 30°C in the presence of [^{15}N]anthranilic acid and [$^{2}H_5$]Trp, the IAA pool became enriched in [^{15}N] from [^{15}N]anthranilic acid well before conversion of [$^{2}H_5$]Trp to [$^{2}H_5$]IAA could be observed. These data show that during normal growth at 30°C, the Trp-I-IAA biosynthesis pathway predominates in these plants. However, when *Lemna* plants are grown at 15°C, label from exogenous [$^{2}H_5$]Trp labels the IAA pool at the same time as, and to a greater extent, than label from [^{15}N]anthranilic acid, indicating that the Trp-D-IAA synthesis pathway is primarily active. Thus, the utilization of the Trp-I-IAA and Trp-D-IAA

synthesis pathways differ at the two growth temperatures (49). In general, a variety of stress situations appear to induce the Trp-D-IAA biosynthesis system.

CONJUGATE BIOSYNTHESIS

All the higher plants tested, as well as many lower plants, conjugate exogenous IAA, primarily to the amino acid aspartate (11, 13). The ability to make IAA-Asp can be enhanced by pretreatment with IAA, and RNA or protein synthesis inhibitors abolish this induction (2). The production of conjugates from applied IAA proved to be a useful assay to establish a relationship between evolutionary development and the complexity of hormone metabolism in land plants (13). These results, together with measurements of the steady state levels of free IAA and IAA metabolites in developmentally staged axenic tissues, revealed that lower plants use a strategy for regulating free IAA that is dependent on *de novo* synthesis of IAA and degradation of existing hormone. As evolution progressed and plants developed more complex vascularization, a strategy for regulating free IAA levels involving conjugate synthesis and hydrolysis became established (Fig. 6). Temporal and spatial patterns of hormone conjugation and hydrolysis, along with differences in conjugate identity and complexity, may reflect the more precise regulation required for growth and development of diverse or complex plant forms (13).

High molecular weight conjugates account for the majority of IAA in many of the tissues examined so far (Fig. 6). A primarily storage role can be envisioned for the considerable amount of IAA-glucan in maize seeds and the IAA-protein or peptide conjugates in bean seeds. Only 2% of the amide conjugates in Arabidopsis seedlings are represented by IAA-Asp and IAA-Glu, the major low molecular weight conjugates, while the majority of the amide conjugates are small peptides of as yet unknown function (29, 62). Antibody to a 3.6 kDa IAA-peptide conjugate found in bean seed cross-reacts with a larger IAA-modified seed protein, IAP1 (66). Cross-reacting proteins were also found in seeds of many other plants, and were found in bean leaf too. The presence of specific peptides or proteins to which IAA is attached may indicate that higher molecular weight conjugates have significance beyond their role in the modulation of IAA levels.

Developmental Controls of IAA Conjugate Synthesis and Hydrolysis

Conjugation of free IAA in maturing seeds serves as a mechanism for storage of IAA in a form that is readily hydrolyzed to provide hormone to the growing shoot upon germination. This strategy is present in gymnosperms as well as monocots and dicots (3), although the particular ester or amide conjugate moieties that are stored in the seed appear to be division specific. In maize and pine, formation of conjugates resumes when the seedling runs out of stored conjugates and the seedling is capable of *de novo* IAA synthesis

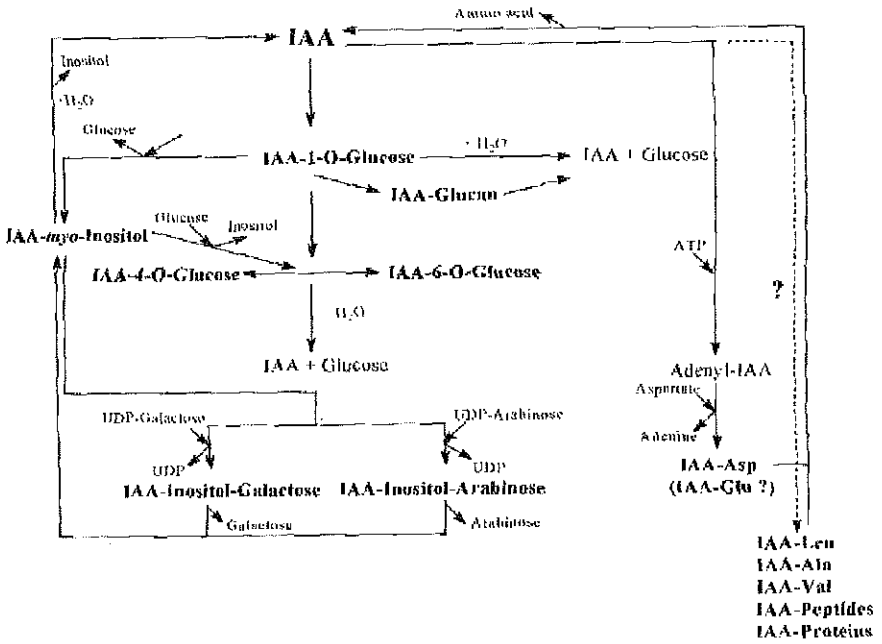

Figure 6. Metabolic transformations between IAA and conjugate pools are shown. Reactions shown have mostly been derived from studies of maize, Arabidopsis and bean, although there is ample evidence for many of these reactions occurring in a diverse array of plant materials (29, 56). The biosynthetic routes, hydrolysis reactions and known inter-conversions are shown. Transacylation from IAA-Inos back to glucose to form the 4-O or 6-O-IAA-gluc are reversible reactions that can lead to the subsequent hydrolysis of either form. The biosynthesis of IAA-Asp by formation of the adenyl-IAA intermediate following auxin induction of the process now appears to be established (58); however, it remains to be determined if the biosynthesis of other IAA-amino acids and higher molecular weight amide conjugates proceed via a similar reaction scheme.

(2, 56). In bean, conjugated forms decrease dramatically only during the first few hours following imbibition (56, 66). *De novo* IAA and IAA-peptide biosynthesis begins in the growing axes while conjugate hydrolysis is still ongoing in the cotyledonary tissue (3, 56, 61, 66). Within a given plant, the type of conjugate formed may be developmentally specific: in pine, ester conjugates predominate in the seed while IAA-Asp, an intermediate in IAA catabolism, is made in the growing shoot (2, 56).

The best-studied systems pertaining to developmental regulation of IAA conjugate formation are the germinating maize kernel (Fig. 7), the germinating pine seed, and developing and germinating bean seed (2, 29, 56). The ester conjugate IAA-Inos is one of the major conjugates in both the kernel and the shoot of maize (2, 56). [^{3}H]IAA-Inos applied to the kernel is transported to the shoot and is, in part, hydrolyzed to yield free [^{3}H]IAA (2, 56). [^{3}H]IAA-Inos-[^{14}C]galactoside applied to the endosperm is also

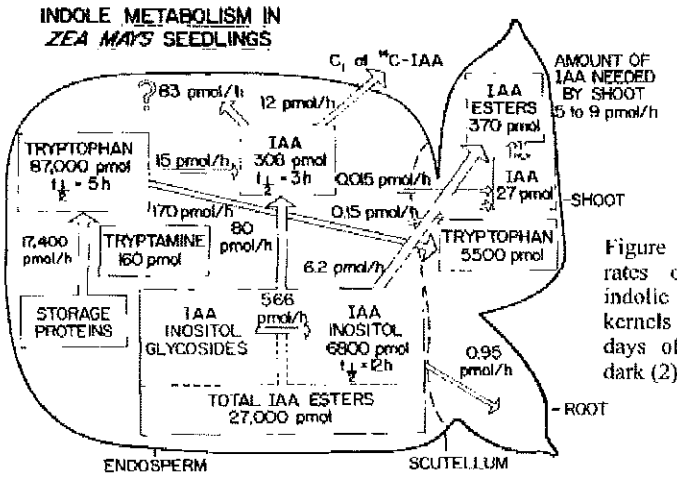

Figure 7. Pool sizes and rates of turnover of the indolic compounds in the kernels of *Zea mays* after 4 days of germination in the dark (2).

transported to the shoot. In these experiments, the double-labeled compound was used to show that hydrolysis of the galactose from the conjugate occurred after the conjugate left the endosperm but before it entered the shoot, presumably in the scutellum. Studies with [^{3}H]IAA-Inos indicated that between 2 to 6 pmol/hr of IAA (Fig . 7) can be supplied to a maize shoot from the kernel and this would be a major source of the estimated 10 pmol/hr required by each shoot (2, 56). As in maize, ester conjugates form the majority of conjugate in the pine seed, and their hydrolysis results in a dramatic increase in free IAA within 48h of imbibition (2, 56). In bean, IAA amide conjugates accumulate throughout seed development, with the major IAA-protein conjugate, IAP1, accumulating late during seed development and rapidly disappearing from both axes and cotyledons during the first day of imbibition (29, 66). Carrot cell cultures differentiating into somatic embryos exhibit a dramatic increase in the rate of conjugate utilization. Order of magnitude decreases in the amide conjugate pool occur over a period of only a few days and this increase in conjugate utilization correlates with a 3-4 times increase in the activity of an IAA-amino acid hydrolase (2, 56).

Biosynthesis of IAA Ester Conjugates

Early biochemical studies in *Zea mays* showed that plants make the IAA-*myo*-inositol family of conjugates by first synthesizing 1-*O*-D-IAA-Gluc from IAA and UDP Gluc, then transferring the IAA to inositol by transacylation (56). IAA-Inos may then be glycosylated to form IAA-Inos-galactoside or IAA-Inos-arabinoside by reaction with the appropriate uridine diphosphosugar. All of the low molecular weight IAA conjugates of *Zea mays* have been shown to be synthesized *in vitro* using enzyme extracts at different levels of purification (56).

A 50.6 kDa UDP-glucosyl transferase catalyzing the formation of IAA-Gluc was purified and characterized from maize, and antibodies raised to the protein were used to select a cDNA clone for the transferase from a maize library (56). Transgenic tomato seedlings expressing the antisense maize sequence had, as might be expected, more roots as well as reduced levels of IAA-Gluc and esterified IAA (22). A 75 kDa tomato protein that cross reacted with antiserum to the maize IAGLU protein was undetectable in these antisense tomato seedlings. By contrast, overexpressing the maize gene in Arabidopsis resulted in a phenotype (diminished root growth and small and/or curly leaves) consistent with reduced free IAA levels. Unexpectedly, the measured IAA levels in these plants were comparable to wild type even though IAA-Gluc levels were increased (J. Ludwig-Muller, personal communication). Arabidopsis UDP glucosyl transferase was identified by an impressive brute force approach combining genomics and biochemistry (23). Putative glucosyltransferase genes encoding enzymes capable of forming glucosyl esters were identified in the Arabidopsis genome. The clones for these genes were expressed in *E. coli* and the products were assayed for activity with a variety of substrates. One clone, UGT84B1, produced the correct products, including the 2-0-, 4-0-, and 6-0-isomers of IAA-Gluc, as identified by mass spectrometry. The expressed protein could use indole proprionic acid and IBA as substrates, but competition studies showed that IAA was preferred. Both IAA and IBA glucose conjugates are formed in plants (62). RT-PCR analyses showed that UGT84B1 is primarily expressed in the siliques, but low expression was detected in inflorescences and roots.

IAA-Gluc is also the precursor to the high molecular weight glucans that form the majority of IAA ester conjugates in the maize kernel (2, 56). In addition, IAA-Gluc undergoes non-enzymatic conversion to 4- and 6-*0*-IAA-Gluc, and enzymatic conversion to di-0-IAA-Gluc in the kernel (2, 56).

Biosynthesis of IAA Amide Conjugates

Mysteriously, and despite numerous attempts, an *in vitro* enzymatic system for synthesis of IAA amide conjugates has never been successfully reconstituted from plants. IAA-Gluc was suggested as a precursor for IAA amide conjugates in plants (23), however transgenic plants overexpressing IAA glucosyl transferase genes either did not accumulate higher levels of amide conjugates or exhibited reduced levels of IAA-Asp or IAA-Glu (22 and J. Ludwig-Muller, personal communication). The recent discovery that at least seven members of the JAR1 family of genes in Arabidopsis were capable of adenylating IAA pointed to a likely pathway for IAA amide conjugation (58). JAR1 proteins belong to the acyl adenylate-forming firefly luciferase superfamily and activate substrates for subsequent biochemical modification by adding AMP to their carboxyl group. One of the Arabidopsis JAR1 family members shows homology to a soybean early auxin inducible protein, GH3, that had been previously described as being

involved in auxin signal transduction based on the phenotype of an activation tagged mutant (35). While it is therefore likely that the mechanism by which inducible IAA-Asp formation proceeds involves adenylation of IAA by a JAR1 family member, we cannot be sure the same mechanism is employed for lower level, constitutive, amide conjugate formation. The redundancy of JAR1-like genes is a possible reason for failure to associate phenotypes with defects in IAA amide conjugate synthesis in the past; however we are now in a position to test the role(s) of these genes in IAA conjugation and in auxin responses.

Hydrolysis of IAA-Conjugates

In 1935, Cholodny (11) showed that application of a water-moistened piece of endosperm to a seedling led to an auxin response whereas alcohol moistened endosperm elicited no response. Thus, Cholodny was the first person to observe enzyme-catalyzed hydrolysis of the IAA conjugates stored in the endosperm. A few years later, Skoog (11) made the important observation that a "seed auxin precursor" moved from the seed to the shoot and that this precursor could be converted by the plant to an active auxin. Extraction of corn shoots or roots with ether for 3 hr at 4°C yielded IAA whereas an extraction with 80% ethanol yielded no IAA. Cold ether does not, in general, inactivate enzymes whereas 80% ethanol does. From these results it was concluded that the tissue was autolyzing in the wet ether, with consequent enzymatic hydrolysis of IAA conjugates to yield free IAA (11). Subsequent work established that the "bound" IAA of the seed could also be released by mild alkaline hydrolysis (11), and that the IAA was not the product of degradation of Trp or storage proteins.

While conjugate hydrolysis in ether-induced autolyzing tissues occurs fairly readily, it has been difficult to obtain *in vitro* hydrolysis of IAA conjugates with purified enzymes. Many commercial proteases and esterases that might be expected to hydrolyze IAA-amino acid conjugates fail to do so (11). *In vitro* hydrolysis has been reported (2, 56), but the hydrolytic enzymes have proven difficult to extract and purify. IAA-inositol hydrolase activity, as well as the co-fractionation of IAGlu synthetase and two enzymes for IAGlu hydrolysis, a 1-*O*-IAGluc hydrolase and a 6-*O*-IAGluc hydrolase, have been reported from maize (2, 56). IAA-Gluc hydrolases have also been found in other plants, including oat, potato and bean (2, 56).

Recently, a genetic approach was used to identify genes involved in hydrolyzing IAA conjugates. The selection based on resistance to the inhibition of root growth by high levels of the IAA conjugates. Those candidates that were resistant to the inhibitory effects of IAA conjugates, but still sensitive to the inhibitory effects of excess IAA were subjected to positional cloning. In this manner, a gene family encoding amidohydrolases was identified. Nineteen IAA-L-amino acid conjugates were tested against the amidohydrolases encoded by this gene family and differing specificities were identified (3, 28). The existence of amidohydrolases with differing

specificities gave impetus to determining the identities of all the IAA conjugates in Arabidopsis (29), and indicates that the less abundant conjugates may play as yet unknown roles in auxin regulation.

Not all the Arabidopsis conjugate resistant mutants identified to date have corresponded to genes encoding amidohydrolases 3). Therefore, these mutants could be useful in determining if the activities of conjugates are solely due to their hydrolysis with release free IAA. Thus, they may ultimately prove to be pivotal in revealing any conjugate functions that do not involve hydrolysis.

The role of IAA-Asp as an intermediate in the catabolism of IAA in some plants (Fig.8) has led to IAA-Asp formation being referred to as an "irreversible" conversion process. This may indeed be true in some plant tissues, and under some conditions. However, a subset of the Arabidopsis amide conjugate hydrolase family has low-level IAA-Asp hydrolase activity (28). In soybean, which stores IAA-Asp and IAA-Glu in its seeds, the

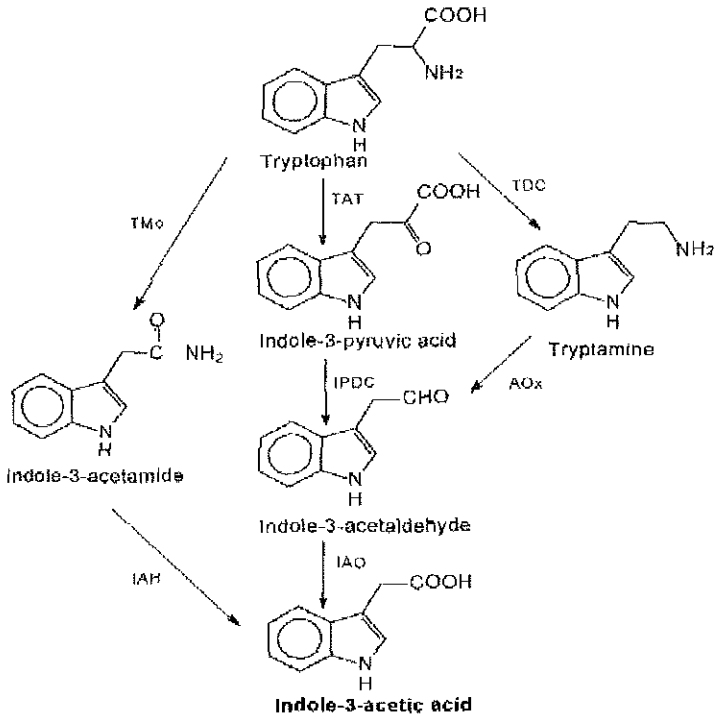

Figure 8. Pathways for the microbial biosynthesis of IAA from tryptophan. Although a number of different enzymes have been proposed for several of the steps, representative ones that have been described are labeled: Tmo: Trp monooxygenase; IAH: indoleacetamide hydrolase; TAT: Trp aminotransferase; IPDC: indole-3-pyruvic acid decarboxylase; IAO: indole-3-acetaldehyde oxidase; IAO; TDC: Trp decarboxylase; Aox: amine oxidase. Little attention has been focused on the Trm pathway in bacteria, although the enzymes are present and bacteria can both make Trm and convert it to IAA added to the culture medium (18).

hypocotyls respond to IAA-L-Asp, but not to IAA-D-Asp, by cell elongation; the growth-inducing activity of IAA-Asp is significant, about 70% of that seen with IAA (2, 56). IAA-Asp is also hydrolyzed, albeit slowly, by bean stems (2, 56).

Microbial Pathways for IAA Biosynthesis

IAA production by plant-associated bacteria has been an important aspect of research on IAA metabolism. It is estimated that as much as 80% of all soil bacteria have the capacity to make IAA, yet this function is generally not essential (18). IAA producing bacteria can be either phytopathogenic or beneficial to plant growth, so the role of IAA production by the microbe isn't completely clear. Wide diversity exists in the specific interactions between plants and bacteria. Within a given bacterial species, there are many variations in metabolic capacity between strains, suggesting a myriad of regulatory mechanisms (18).

The IAA biosynthetic genes encoded by the crown gall forming bacteria *Agrobacterium tumefaciens* have proven very useful in refining hypotheses about auxin action in plants (25). *Agrobacterium tumefaciens* transfers genetic material (a fragment, termed T-DNA, of the tumor inducing Ti plasmid) from the infecting bacteria into the host plant. Encoded within the transferred DNA are genes for the enzymes Trp monooxygenase and indoleacetamide hydrolase (18), which carry out the conversion of Trp to indoleacetamide and the hydrolysis of the indoleacetamide to IAA by the infected plant cell (Fig. 8). Ectopic overexpression of these genes in a plant independent of other Ti plasmid genes that induce tumor formation, has been useful in characterizing the effect of increased IAA production *in planta*. In addition to confirming many of the observations that were made in classic hormone application studies (25), stable isotope labeling studies in such lines have revealed cross talk between IAA and cytokinin homeostasis pathways (15).

Another gall forming bacterium, *Pseudomonas syringae* pv. *Savastanoi*, uses the identical pathway for IAA production (2); however, in this case no genetic material is transferred and the bacteria themselves produce high levels of IAA. Pathogenic *Pseudomonas* also have the capacity to form the novel conjugate of IAA, IAA-Lys, as well as its α-N-acetyl derivative (2). Although IAA-Lys formation reduces the pool of free IAA produced by the bacteria by about 30%, the role of these conjugates in gall formation has not been established.

Nonpathogenic, plant-associated bacteria that inhabit the rhizosphere appear to enhance plant growth by a variety of means, including secretion of IAA into the rhizosphere. Mutations in the *Pseudomonas putida* gene encoding indole pyruvate decarboxylase, *ipdc* (Fig. 8), render the strain unable to stimulate root growth compared to wild type strains (46). Some strains of *Erwinia herbicola* cause crown and root galls on *Gypsophila paniculata* (2). The strains of *Erwinia* that are pathogenic and form such

galls have the capacity to make most of their IAA by the same indoleacetamide pathway as *Pseudomonas* and *Agrobacterium* T-DNA transformed plant cells. Mutation analysis indicated that the capacity for gall formation requires the indoleacetamide pathway (18). *Erwinia* mutants defective in the indoleacetamide pathway were unable to form galls, but were still capable of producing IAA via the indole-3-pyruvic acid pathway, albeit at lower levels (18). The *ipdc* promoter in *Pseudomonas putida* strains appears to be regulated by both Trp and the stationary phase sigma factor RpoS (47) while the indoleacetamide pathway appears to be constitutive in most systems examined (18). Overexpression of rpoS in *Enterobacter* strains results in accumulation of IAA and enhanced root growth when the strain is incubated with seedlings (47).

Some soil bacteria have the capacity to hydrolyze IAA conjugates from plants (9). Indeed, the gene for a specific IAA-Asp hydrolase (9) has provided a useful tool for studies of the compound's role *in planta* (63). Many of the nonpathogenic strains of IAA-producing bacteria are saprophytic epiphytes that are widespread in nature. Their presence in plants grown in non-sterile conditions is a potential complication for IAA biosynthesis studies because bacterial IAA production by the endophyte could be considerably greater than plant IAA production rates.

Recently, cyanobacteria, the photosynthetic bacteria that are the progenitors of chloroplasts, have been shown to produce IAA by a Trp-D-IAA synthesis pathway, probably via indole-3-pyruvic acid (Figs. 4 and 8) (55). In higher plants, Trp biosynthetic enzymes are chloroplast localized, as are some enzymes in Trp-D IAA synthesis pathways (e.g. CYP79B2 and CYP79B3) (12).

IAA CATABOLISM

Oxidative catabolism of IAA is generally considered to be the chemical modification of the indole nucleus or side chain that results in loss of auxin activity. As far as we know, it is the only truly irreversible step regulating IAA levels. Studies of IAA catabolism predate even the identification of IAA as a ubiquitous auxin in plants, and reports of peroxidase enzymes that catalyze the oxidative decarboxylation of IAA are numerous (2). For many years this reaction was generally assumed to be the physiological route for oxidative catabolism. Although a copious amount of information is available on the peroxidation of IAA *in vitro*, the products formed are not usually found in plants in significant amounts. Also, the rate of peroxidation of IAA applied to plants, as measured by CO_2 evolution, is usually only a small fraction of the measured rate of IAA turnover. These same problems, low rates of CO_2 evolution and failure to identify the oxidative products in normal plant materials, also apply to recent studies of ascorbic acid oxidase catabolism of IAA in maize roots (24). These considerations, coupled with studies of plants over and under expressing specific peroxidases (39), suggest

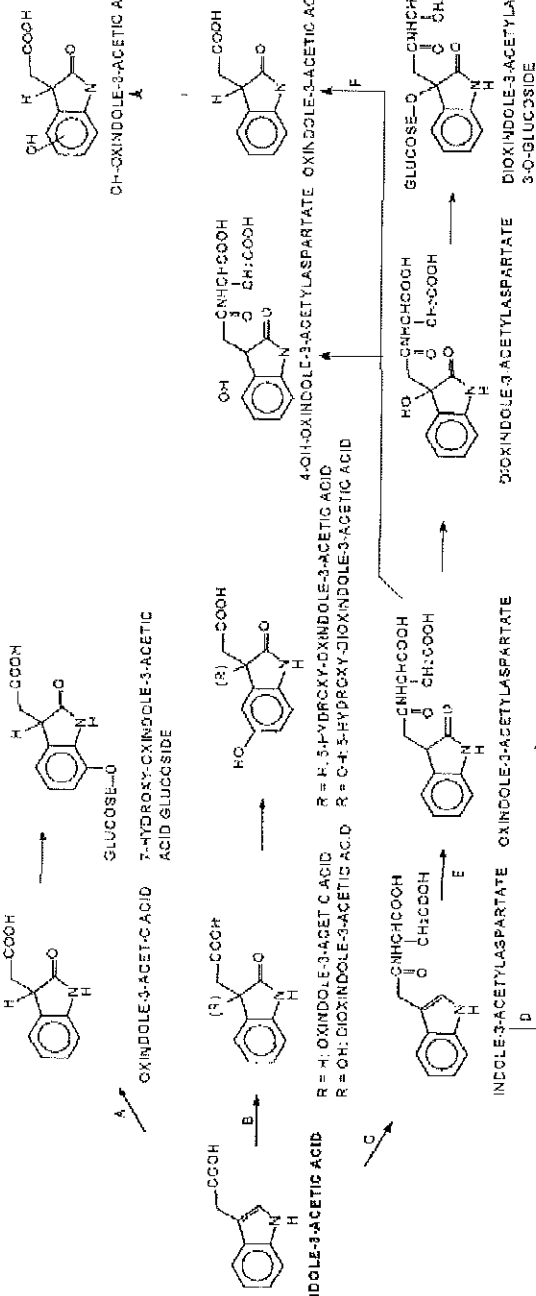

Figure 9. Oxidation routes for the catabolism of IAA known to occur in plants, based on the identification of the oxidation products from untreated plant materials as well as from isotopic labeling studies (39). In some cases, the responsible enzyme(s) have also been partially purified (2). Each of these pathways have been primarily studied in only one or a few species: A: *Zea mays*; B: *Oryza sativa*; C: most plants, including *Zea mays*, *Pisum sativum* and Arabidopsis; D: *Pinus sylvestris*; E: *Vicia faba*, *Dalbergia dolechopetala*; F: *Lycopersicon esculentum*.

that this is not likely a major route for IAA catabolism *in vivo* under most conditions. More recently a new pathway of IAA catabolism, non-decarboxylative oxidation with retention of the carboxyl side chain and oxidation of the indole nucleus, has been identified (2, 39) and shown to occur in a number of plant species.

The Oxindole-3-Acetic Acid/Dioxindole-3-Acetic Acid Pathway

The direct ring oxidation pathway for IAA catabolism has been the subject of detailed study in several plant species including rice, corn, and broad bean (29). Based on simple colorimetric assays, the first product in this pathway (Fig. 9), OxIAA, also occurs in germinating seeds of *Brassica rapa*, and developing seeds of *Ribes rubrum* (2). *Oryza sativa*, rice, is interesting in that it is the only plant known to contain both OxIAA and DiOxIAA (2), and was also shown to have the 5-hydroxy analogs of OxIAA and DiOxIAA. OxIAA has been shown not to be an intermediate or a substrate for the peroxidase pathway (2), so the pathways are independent.

The first report of IAA oxidation to oxindole-3-acetic acid (OxIAA) was in the basidiomycete *Hygrophorus conicus* (2). OxIAA and DiOxIAA were later found to be synthesized by *Zea mays* and *Vicia faba*, respectively, following feeding of 1-[^{14}C]-IAA (2). Isotope dilution experiments (2) showed that OxIAA was a naturally occurring compound in *Zea mays* endosperm and shoot tissues, occurring in amounts of 357 pmol per endosperm and 47 pmol per shoot, about the level of free IAA in these tissues. In *Vicia faba*, DiOxIAA was estimated by UV measurements to be 1 μM in roots (2).

OxIAA is further metabolized in maize by hydroxylation at the 7 position, and by glucose addition to form 7-OH-OxIAA-glucoside (2). Isotope dilution assays have shown that the 7-OH-OxIAA-glucoside is a naturally occurring compound in corn in amounts of 62 pmol per shoot and 4,800 pmol per endosperm. *Vicia faba* is also reported to form a glucose derivative of DiOx-IAA-aspartate (2). 7-OH-OxIAA has also been identified as a catabolite of IAA in germinating kernels of *Zea mays*. It was found to be present at 3,100 pmol in endosperm and has been shown to be an intermediate in the synthesis of 7-OH-OxIAA-glucoside (2). Since the glucoside is present in much higher amounts than IAA, OxIAA, and somewhat higher than 7-OH-OxIAA, it has been speculated that it might be the form that accumulates in vacuoles (2). The further metabolism of 7-OH-OxIAA and 7-OH-OxIAA-glucoside has not been studied except that 5-[^{3}H]-7-OH-OxIAA loses tritium to water upon further enzymatic oxidation (2). This implies a second oxidation of the benzenoid ring leading to a highly unstable dioxindole.

Much less is known about the enzymology of the OxIAA and DiOxIAA pathways than has accumulated about the action of peroxidase on IAA. This is due to its more recent discovery, the limited availability of enzymes and substrates, and the difficulty of establishing robust *in vitro* assays. In *Zea*

mays, the rate of oxidation of IAA to OxIAA has been measured in shoot, root, scutellar, and endosperm tissues at 1-10 pmol h^{-1} mg $protein^{-1}$. The enzyme that carries out this reaction is soluble, of high molecular weight and clearly different from lipoxygenase or peroxidase (2). Enzyme activity was reduced by 90 per cent when assayed under argon, indicating an oxygen requirement. Enzyme activity was stimulated up to ten fold by addition of an ionic detergent extract of corn tissue. A heat stable lipophylic component of these extracts was identified as the factor that increased enzyme activity when added to buffer extracted enzyme. This heat stable factor could be replaced by linolenic, linoleic, or arachidonic acid (2), which could be acting as co-substrates.

IAA-Asp Oxidation

A specific route of IAA-conjugate oxidation was demonstrated in work that showed that in *Vicia* seedlings, IAA-Asp is oxidized to di-Ox-IAA-aspartate without prior hydrolysis to the free acid (2). As with IAA oxidation, once IAA-Asp oxidation occurs, the product is glycosylated to form the 3-(*O*-β-glucosyl) derivative. IAA-Asp could be oxidized by peroxidase, but onl when peroxide was added to the reaction mixture (2). The product of this oxidation was 2-OH-Ox-IAAsp, thus, the reaction with peroxidase and H_2O_2 yields a different product from that isolated from the plant. In *Populus*, IAAsp, OxIAAsp and ring hydroxylated products of OxIAAsp are the major products formed after feeding with IAA (2). In Scots pine, IAA is oxidized to OxIAA as well as conjugated to IAAsp. These compounds are further metabolized to glucopyranosyl-1-N-IAAsp and glucosyl-1-N-IAA. Several other species have been studied (2, 29) and, in general, two basic strategies appear to be followed with individual differences dependent on species: IAA is either oxidized and glycosylated directly, or these processes are carried out following conjugation with aspartate.

SUMMARY

Through the marriage of molecular genetics to highly sensitive analytical methods, a wealth of information about IAA homeostasis pathways has recently been uncovered. Redundancy and plasticity are becoming the two overriding themes to the network of pathways that determine how much auxin is present in a plant at any given time (12, 40). There are still more players to be identified, from the enzymes that carry out the individual steps in a pathway to the regulatory components that render these pathways so responsive. Genome level approaches should yield more new information in this regard and, activation tagging[2] is a new method that holds promise for

[2] Activation tagging refers to transgenic plants in which a DNA sequence that activates transcription of downstream sequences has been randomly inserted into the genome, thus randomly activating nearby genes. Mutant phenotypes may result from the activation of said gene and can provide clues to the function of these genes.

identifying genes in families that would be masked in a conventional genetic screen. Rapid and ultrasensitive assays for high throughput auxin analyses are in the pipeline, and should greatly facilitate genetic discoveries.

It is now well established that a plant's auxin metabolic response is intricately tied to other pathways that respond to a wide variety of signals, including temperature, light, jasmonic acid, other plant hormones and second messengers. The molecular intermediaries in these interactions are just beginning to be discovered and protein phosphorylation is emerging as a central component (59). Genetic approaches and global profiling strategies will be particularly useful in assessing the effect of mutations in one pathway upon interacting pathways, both during normal plant growth and development as well as during periods of pathogen infection or abiotic stress.

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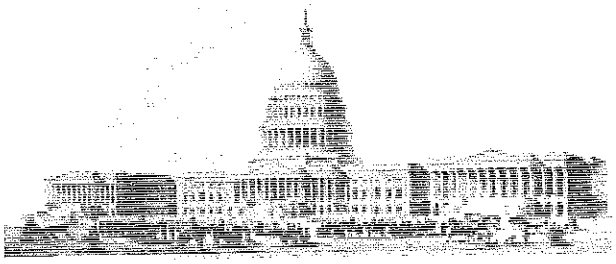

§ 136a. Registration of pesticides

How Current is This?

(a) Requirement of registration

Except as provided by this subchapter, no person in any State may distribute or sell to any person any pesticide that is not registered under this subchapter. To the extent necessary to prevent unreasonable adverse effects on the environment, the Administrator may by regulation limit the distribution, sale, or use in any State of any pesticide that is not registered under this subchapter and that is not the subject of an experimental use permit under section 136c of this title or an emergency exemption under section 136p of this title.

(b) Exemptions

A pesticide which is not registered with the Administrator may be transferred if—

(1) the transfer is from one registered establishment to another registered establishment operated by the same producer solely for packaging at the second establishment or for use as a constituent part of another pesticide produced at the second establishment; or

(2) the transfer is pursuant to and in accordance with the requirements of an experimental use permit.

(c) Procedure for registration

(1) Statement required

Each applicant for registration of a pesticide shall file with the Administrator a statement which includes—

(A) the name and address of the applicant and of any other person whose name will appear on the labeling;

(B) the name of the pesticide;

(C) a complete copy of the labeling of the pesticide, a statement of all claims to be made for it, and any directions for its use;

(D) the complete formula of the pesticide;

(E) a request that the pesticide be classified for general use or for restricted use,

or for both; and

(F) except as otherwise provided in paragraph (2)(D), if requested by the Administrator, a full description of the tests made and the results thereof upon which the claims are based, or alternatively a citation to data that appear in the public literature or that previously had been submitted to the Administrator and that the Administrator may consider in accordance with the following provisions:

(i) With respect to pesticides containing active ingredients that are initially registered under this subchapter after September 30, 1978, data submitted to support the application for the original registration of the pesticide, or an application for an amendment adding any new use to the registration and that pertains solely to such new use, shall not, without the written permission of the original data submitter, be considered by the Administrator to support an application by another person during a period of ten years following the date the Administrator first registers the pesticide, except that such permission shall not be required in the case of defensive data.

(ii) The period of exclusive data use provided under clause (i) shall be extended 1 additional year for each 3 minor uses registered after August 3, 1996, and within 7 years of the commencement of the exclusive use period, up to a total of 3 additional years for all minor uses registered by the Administrator if the Administrator, in consultation with the Secretary of Agriculture, determines that, based on information provided by an applicant for registration or a registrant, that—

(I) there are insufficient efficacious alternative registered pesticides available for the use;

(II) the alternatives to the minor use pesticide pose greater risks to the environment or human health;

(III) the minor use pesticide plays or will play a significant part in managing pest resistance; or

(IV) the minor use pesticide plays or will play a significant part in an integrated pest management program.

The registration of a pesticide for a minor use on a crop grouping established by the Administrator shall be considered for purposes of this clause 1 minor use for each representative crop for which data are provided in the crop grouping. Any additional exclusive use period under this clause shall be modified as appropriate or terminated if the registrant voluntarily cancels the product or deletes from the registration the minor uses which formed the basis for the extension of the additional exclusive use period or

if the Administrator determines that the registrant is not actually marketing the product for such minor uses.

(iii) Except as otherwise provided in clause (i), with respect to data submitted after December 31, 1969, by an applicant or registrant to support an application for registration, experimental use permit, or amendment adding a new use to an existing registration, to support or maintain in effect an existing registration, or for reregistration, the Administrator may, without the permission of the original data submitter, consider any such item of data in support of an application by any other person (hereinafter in this subparagraph referred to as the "applicant") within the fifteen-year period following the date the data were originally submitted only if the applicant has made an offer to compensate the original data submitter and submitted such offer to the Administrator accompanied by evidence of delivery to the original data submitter of the offer. The terms and amount of compensation may be fixed by agreement between the original data submitter and the applicant, or, failing such agreement, binding arbitration under this subparagraph. If, at the end of ninety days after the date of delivery to the original data submitter of the offer to compensate, the original data submitter and the applicant have neither agreed on the amount and terms of compensation nor on a procedure for reaching an agreement on the amount and terms of compensation, either person may initiate binding arbitration proceedings by requesting the Federal Mediation and Conciliation Service to appoint an arbitrator from the roster of arbitrators maintained by such Service. The procedure and rules of the Service shall be applicable to the selection of such arbitrator and to such arbitration proceedings, and the findings and determination of the arbitrator shall be final and conclusive, and no official or court of the United States shall have power or jurisdiction to review any such findings and determination, except for fraud, misrepresentation, or other misconduct by one of the parties to the arbitration or the arbitrator where there is a verified complaint with supporting affidavits attesting to specific instances of such fraud, misrepresentation, or other misconduct. The parties to the arbitration shall share equally in the payment of the fee and expenses of the arbitrator. If the Administrator determines that an original data submitter has failed to participate in a procedure for reaching an agreement or in an arbitration proceeding as required by this subparagraph, or failed to comply with the terms of an agreement or arbitration decision concerning compensation under this subparagraph, the original data submitter shall forfeit the right to compensation for the use of the data in support of the application. Notwithstanding any other provision of this subchapter, if the Administrator determines that an applicant has failed to participate in a procedure for reaching an agreement or in an arbitration proceeding as required by this subparagraph, or failed to comply with the terms of an agreement or

arbitration decision concerning compensation under this subparagraph, the Administrator shall deny the application or cancel the registration of the pesticide in support of which the data were used without further hearing. Before the Administrator takes action under either of the preceding two sentences, the Administrator shall furnish to the affected person, by certified mail, notice of intent to take action and allow fifteen days from the date of delivery of the notice for the affected person to respond. If a registration is denied or canceled under this subparagraph, the Administrator may make such order as the Administrator deems appropriate concerning the continued sale and use of existing stocks of such pesticide. Registration action by the Administrator shall not be delayed pending the fixing of compensation.

(iv) After expiration of any period of exclusive use and any period for which compensation is required for the use of an item of data under clauses (i), (ii), and (iii), the Administrator may consider such item of data in support of an application by any other applicant without the permission of the original data submitter and without an offer having been received to compensate the original data submitter for the use of such item of data.

(v) The period of exclusive use provided under clause (ii) shall not take effect until 1 year after August 3, 1996, except where an applicant or registrant is applying for the registration of a pesticide containing an active ingredient not previously registered.

(vi) With respect to data submitted after August 3, 1996, by an applicant or registrant to support an amendment adding a new use to an existing registration that does not retain any period of exclusive use, if such data relates solely to a minor use of a pesticide, such data shall not, without the written permission of the original data submitter, be considered by the Administrator to support an application for a minor use by another person during the period of 10 years following the date of submission of such data. The applicant or registrant at the time the new minor use is requested shall notify the Administrator that to the best of their knowledge the exclusive use period for the pesticide has expired and that the data pertaining solely to the minor use of a pesticide is eligible for the provisions of this paragraph. If the minor use registration which is supported by data submitted pursuant to this subsection is voluntarily canceled or if such data are subsequently used to support a nonminor use, the data shall no longer be subject to the exclusive use provisions of this clause but shall instead be considered by the Administrator in accordance with the provisions of clause (i), as appropriate.

(G) If the applicant is requesting that the registration or amendment to the registration of a pesticide be expedited, an explanation of the basis for the request must be submitted, in accordance with paragraph (10) of this subsection.

(2) Data in support of registration

(A) In general

The Administrator shall publish guidelines specifying the kinds of information which will be required to support the registration of a pesticide and shall revise such guidelines from time to time. If thereafter the Administrator requires any additional kind of information under subparagraph (B) of this paragraph, the Administrator shall permit sufficient time for applicants to obtain such additional information. The Administrator, in establishing standards for data requirements for the registration of pesticides with respect to minor uses, shall make such standards commensurate with the anticipated extent of use, pattern of use, the public health and agricultural need for such minor use, and the level and degree of potential beneficial or adverse effects on man and the environment. The Administrator shall not require a person to submit, in relation to a registration or reregistration of a pesticide for minor agricultural use under this subchapter, any field residue data from a geographic area where the pesticide will not be registered for such use. In the development of these standards, the Administrator shall consider the economic factors of potential national volume of use, extent of distribution, and the impact of the cost of meeting the requirements on the incentives for any potential registrant to undertake the development of the required data. Except as provided by section 136h of this title, within 30 days after the Administrator registers a pesticide under this subchapter the Administrator shall make available to the public the data called for in the registration statement together with such other scientific information as the Administrator deems relevant to the Administrator's decision.

(B) Additional data

(i) If the Administrator determines that additional data are required to maintain in effect an existing registration of a pesticide, the Administrator shall notify all existing registrants of the pesticide to which the determination relates and provide a list of such registrants to any interested person.

(ii) Each registrant of such pesticide shall provide evidence within ninety days after receipt of notification that it is taking appropriate steps to secure the additional data that are required. Two or more registrants may agree to develop jointly, or to share in the cost of developing, such data if they agree

and advise the Administrator of their intent within ninety days after notification. Any registrant who agrees to share in the cost of producing the data shall be entitled to examine and rely upon such data in support of maintenance of such registration. The Administrator shall issue a notice of intent to suspend the registration of a pesticide in accordance with the procedures prescribed by clause (iv) if a registrant fails to comply with this clause.

(iii) If, at the end of sixty days after advising the Administrator of their agreement to develop jointly, or share in the cost of developing, data, the registrants have not further agreed on the terms of the data development arrangement or on a procedure for reaching such agreement, any of such registrants may initiate binding arbitration proceedings by requesting the Federal Mediation and Conciliation Service to appoint an arbitrator from the roster of arbitrators maintained by such Service. The procedure and rules of the Service shall be applicable to the selection of such arbitrator and to such arbitration proceedings, and the findings and determination of the arbitrator shall be final and conclusive, and no official or court of the United States shall have power or jurisdiction to review any such findings and determination, except for fraud, misrepresentation, or other misconduct by one of the parties to the arbitration or the arbitrator where there is a verified complaint with supporting affidavits attesting to specific instances of such fraud, misrepresentation, or other misconduct. All parties to the arbitration shall share equally in the payment of the fee and expenses of the arbitrator. The Administrator shall issue a notice of intent to suspend the registration of a pesticide in accordance with the procedures prescribed by clause (iv) if a registrant fails to comply with this clause.

(iv) Notwithstanding any other provision of this subchapter, if the Administrator determines that a registrant, within the time required by the Administrator, has failed to take appropriate steps to secure the data required under this subparagraph, to participate in a procedure for reaching agreement concerning a joint data development arrangement under this subparagraph or in an arbitration proceeding as required by this subparagraph, or to comply with the terms of an agreement or arbitration decision concerning a joint data development arrangement under this subparagraph, the Administrator may issue a notice of intent to suspend such registrant's registration of the pesticide for which additional data is required. The Administrator may include in the notice of intent to suspend such provisions as the Administrator deems appropriate concerning the continued sale and use of existing stocks of such pesticide. Any suspension proposed under this subparagraph shall become final and effective at the end of thirty days from receipt by the registrant of the notice of intent to suspend, unless during that time a request for hearing is made by a person

adversely affected by the notice or the registrant has satisfied the Administrator that the registrant has complied fully with the requirements that served as a basis for the notice of intent to suspend. If a hearing is requested, a hearing shall be conducted under section 136d (d) of this title. The only matters for resolution at that hearing shall be whether the registrant has failed to take the action that served as the basis for the notice of intent to suspend the registration of the pesticide for which additional data is required, and whether the Administrator's determination with respect to the disposition of existing stocks is consistent with this subchapter. If a hearing is held, a decision after completion of such hearing shall be final. Notwithstanding any other provision of this subchapter, a hearing shall be held and a determination made within seventy-five days after receipt of a request for such hearing. Any registration suspended under this subparagraph shall be reinstated by the Administrator if the Administrator determines that the registrant has complied fully with the requirements that served as a basis for the suspension of the registration.

(v) Any data submitted under this subparagraph shall be subject to the provisions of paragraph (1)(D). Whenever such data are submitted jointly by two or more registrants, an agent shall be agreed on at the time of the joint submission to handle any subsequent data compensation matters for the joint submitters of such data.

(vi) Upon the request of a registrant the Administrator shall, in the case of a minor use, extend the deadline for the production of residue chemistry data under this subparagraph for data required solely to support that minor use until the final deadline for submission of data under section 136a–1 of this title for the other uses of the pesticide established as of August 3, 1996, if—

(I) the data to support other uses of the pesticide on a food are being provided;

(II) the registrant, in submitting a request for such an extension, provides a schedule, including interim dates to measure progress, to assure that the data production will be completed before the expiration of the extension period;

(III) the Administrator has determined that such extension will not significantly delay the Administrator's schedule for issuing a reregistration eligibility determination required under section 136a–1 of this title; and

(IV) the Administrator has determined that based on existing data, such extension would not significantly increase the risk of any unreasonable adverse effect on the environment. If the Administrator grants an

extension under this clause, the Administrator shall monitor the development of the data and shall ensure that the registrant is meeting the schedule for the production of the data. If the Administrator determines that the registrant is not meeting or has not met the schedule for the production of such data, the Administrator may proceed in accordance with clause (iv) regarding the continued registration of the affected products with the minor use and shall inform the public of such action. Notwithstanding the provisions of this clause, the Administrator may take action to modify or revoke the extension under this clause if the Administrator determines that the extension for the minor use may cause an unreasonable adverse effect on the environment. In such circumstance, the Administrator shall provide, in writing to the registrant, a notice revoking the extension of time for submission of data. Such data shall instead be due in accordance with the date established by the Administrator for the submission of the data.

(vii) If the registrant does not commit to support a specific minor use of the pesticide, but is supporting and providing data in a timely and adequate fashion to support uses of the pesticide on a food, or if all uses of the pesticide are nonfood uses and the registrant does not commit to support a specific minor use of the pesticide but is supporting and providing data in a timely and adequate fashion to support other nonfood uses of the pesticide, the Administrator, at the written request of the registrant, shall not take any action pursuant to this clause in regard to such unsupported minor use until the final deadline established as of August 3, 1996, for the submission of data under section 136a–1 of this title for the supported uses identified pursuant to this clause unless the Administrator determines that the absence of the data is significant enough to cause human health or environmental concerns. On the basis of such determination, the Administrator may refuse the request for extension by the registrant. Upon receipt of the request from the registrant, the Administrator shall publish in the Federal Register a notice of the receipt of the request and the effective date upon which the uses not being supported will be voluntarily deleted from the registration pursuant to section 136d (f)(1) of this title. If the Administrator grants an extension under this clause, the Administrator shall monitor the development of the data for the uses being supported and shall ensure that the registrant is meeting the schedule for the production of such data. If the Administrator determines that the registrant is not meeting or has not met the schedule for the production of such data, the Administrator may proceed in accordance with clause (iv) of this subparagraph regarding the continued registration of the affected products with the minor and other uses and shall inform the public of such action in accordance with section 136d (f)(2) of

this title. Notwithstanding the provisions of this clause, the Administrator may deny, modify, or revoke the temporary extension under this subparagraph if the Administrator determines that the continuation of the minor use may cause an unreasonable adverse effect on the environment. In the event of modification or revocation, the Administrator shall provide, in writing, to the registrant a notice revoking the temporary extension and establish a new effective date by which the minor use shall be deleted from the registration.

(viii)

(I) If data required to support registration of a pesticide under subparagraph (A) is requested by a Federal or State regulatory authority, the Administrator shall, to the extent practicable, coordinate data requirements, test protocols, timetables, and standards of review and reduce burdens and redundancy caused to the registrant by multiple requirements on the registrant.

(II) The Administrator may enter into a cooperative agreement with a State to carry out subclause (I).

(III) Not later than 1 year after August 3, 1996, the Administrator shall develop a process to identify and assist in alleviating future disparities between Federal and State data requirements.

(C) Simplified procedures

Within nine months after September 30, 1978, the Administrator shall, by regulation, prescribe simplified procedures for the registration of pesticides, which shall include the provisions of subparagraph (D) of this paragraph.

(D) Exemption

No applicant for registration of a pesticide who proposes to purchase a registered pesticide from another producer in order to formulate such purchased pesticide into the pesticide that is the subject of the application shall be required to—

(i) submit or cite data pertaining to such purchased product; or

(ii) offer to pay reasonable compensation otherwise required by paragraph (1)(D) of this subsection for the use of any such data.

(E) Minor use waiver

In handling the registration of a pesticide for a minor use, the Administrator may waive otherwise applicable data requirements if the Administrator determines that the absence of such data will not prevent the Administrator from determining—

(i) the incremental risk presented by the minor use of the pesticide; and

(ii) that such risk, if any, would not be an unreasonable adverse effect on the environment.

(3) Application

(A) In general

The Administrator shall review the data after receipt of the application and shall, as expeditiously as possible, either register the pesticide in accordance with paragraph (5), or notify the applicant of the Administrator's determination that it does not comply with the provisions of the subchapter in accordance with paragraph (6).

(B) Identical or substantially similar

(i) The Administrator shall, as expeditiously as possible, review and act on any application received by the Administrator that—

(I) proposes the initial or amended registration of an end-use pesticide that, if registered as proposed, would be identical or substantially similar in composition and labeling to a currently-registered pesticide identified in the application, or that would differ in composition and labeling from such currently-registered pesticide only in ways that would not significantly increase the risk of unreasonable adverse effects on the environment; or

(II) proposes an amendment to the registration of a registered pesticide that does not require scientific review of data.

(ii) In expediting the review of an application for an action described in *clause (i), the Administrator shall*—

(I) within 45 days after receiving the application, notify the registrant whether or not the application is complete and, if the application is found to be incomplete, reject the application;

(II) within 90 days after receiving a complete application, notify the registrant if the application has been granted or denied; and

(III) if the application is denied, notify the registrant in writing of the specific reasons for the denial of the application.

(C) Minor use registration

(i) The Administrator shall, as expeditiously as possible, review and act on any complete application—

(I) that proposes the initial registration of a new pesticide active ingredient if the active ingredient is proposed to be registered solely for *minor uses, or proposes a registration amendment solely for minor uses* to an existing registration; or

(II) for a registration or a registration amendment that proposes significant minor uses.

(ii) For the purposes of clause (i)—

(I) the term "as expeditiously as possible" means that the Administrator shall, to the greatest extent practicable, complete a review and evaluation of all data, submitted with a complete application, within 12 months after the submission of the complete application, and the failure of the Administrator to complete such a review and evaluation under clause (i) shall not be subject to judicial review; and

(II) the term "significant minor uses" means 3 or more minor uses proposed for every nonminor use, a minor use that would, in the judgment of the Administrator, serve as a replacement for any use which has been canceled in the 5 years preceding the receipt of the application, or a minor use that in the opinion of the Administrator would avoid the reissuance of an emergency exemption under section 136p of this title for that minor use.

(D) Adequate time for submission of minor use data

If a registrant makes a request for a minor use waiver, regarding data required by the Administrator, pursuant to paragraph (2)(E), and if the Administrator denies in whole or in part such data waiver request, the registrant shall have a full-time period for providing such data. For purposes of this subparagraph, the term "full-time period" means the time period originally established by the Administrator for submission of such data, beginning with the date of receipt by the registrant of the Administrator's notice of denial.

(4) Notice of application

The Administrator shall publish in the Federal Register, promptly after receipt of the statement and other data required pursuant to paragraphs (1) and (2), a notice of each application for registration of any pesticide if it contains any new active ingredient or if it would entail a changed use pattern. The notice shall provide for a period of 30 days in which any Federal agency or any other interested person may comment.

(5) Approval of registration

The Administrator shall register a pesticide if the Administrator determines that, when considered with any restrictions imposed under subsection (d) of this section—

(A) its composition is such as to warrant the proposed claims for it;

(B) its labeling and other material required to be submitted comply with the requirements of this subchapter;

(C) it will perform its intended function without unreasonable adverse effects on the environment; and

(D) when used in accordance with widespread and commonly recognized practice it will not generally cause unreasonable adverse effects on the environment.

The Administrator shall not make any lack of essentiality a criterion for denying registration of any pesticide. Where two pesticides meet the requirements of this paragraph, one should not be registered in preference to the other. In considering an application for the registration of a pesticide, the Administrator may waive data requirements pertaining to efficacy, in which event the Administrator may register the pesticide without determining that the pesticide's composition is such as to warrant proposed claims of efficacy. If a pesticide is found to be efficacious by any State under section 136v (c) of this title, a presumption is established that the Administrator shall waive data requirements pertaining to efficacy for use of the pesticide in such State.

(6) Denial of registration

If the Administrator determines that the requirements of paragraph (5) for registration are not satisfied, the Administrator shall notify the applicant for registration of the Administrator's determination and of the Administrator's reasons (including the factual basis) therefor, and that, unless the applicant corrects the conditions and notifies the Administrator thereof during the 30-day period beginning with the day after the date on which the applicant receives the notice, the Administrator may refuse to register the pesticide. Whenever the Administrator

refuses to register a pesticide, the Administrator shall notify the applicant of the Administrator's decision and of the Administrator's reasons (including the factual basis) therefor. The Administrator shall promptly publish in the Federal Register notice of such denial of registration and the reasons therefor. Upon such notification, the applicant for registration or other interested person with the concurrence of the applicant shall have the same remedies as provided for in section 136d of this title.

(7) Registration under special circumstances

Notwithstanding the provisions of paragraph (5)—

(A) The Administrator may conditionally register or amend the registration of a *pesticide if the Administrator determines that*

(i) the pesticide and proposed use are identical or substantially similar to any currently registered pesticide and use thereof, or differ only in ways that would not significantly increase the risk of unreasonable adverse effects on the environment, and

(ii) approving the registration or amendment in the manner proposed by the applicant would not significantly increase the risk of any unreasonable adverse effect on the environment. An applicant seeking conditional registration or amended registration under this subparagraph shall submit such data as would be required to obtain registration of a similar pesticide under paragraph (5). If the applicant is unable to submit an item of data because it has not yet been generated, the Administrator may register or amend the registration of the pesticide under such conditions as will require the submission of such data not later than the time such data are required to be submitted with respect to similar pesticides already registered under this subchapter.

(B) The Administrator may conditionally amend the registration of a pesticide to permit additional uses of such pesticide notwithstanding that data concerning the pesticide may be insufficient to support an unconditional amendment, if the Administrator determines that

(i) the applicant has submitted satisfactory data pertaining to the proposed additional use, and

(ii) amending the registration in the manner proposed by the applicant would not significantly increase the risk of any unreasonable adverse effect on the environment. Notwithstanding the foregoing provisions of this subparagraph, no registration of a pesticide may be amended to permit an additional use of such pesticide if the Administrator has issued a notice

stating that such pesticide, or any ingredient thereof, meets or exceeds risk criteria associated in whole or in part with human dietary exposure enumerated in regulations issued under this subchapter, and during the pendency of any risk-benefit evaluation initiated by such notice, if

(I) the additional use of such pesticide involves a major food or feed crop, or

(II) the additional use of such pesticide involves a minor food or feed crop and the Administrator determines, with the concurrence of the Secretary of Agriculture, there is available an effective alternative pesticide that does not meet or exceed such risk criteria. An applicant seeking amended registration under this subparagraph shall submit such data as would be required to obtain registration of a similar pesticide under paragraph (5). If the applicant is unable to submit an item of data (other than data pertaining to the proposed additional use) because it has not yet been generated, the Administrator may amend the registration under such conditions as will require the submission of such data not later than the time such data are required to be submitted with respect to similar pesticides already registered under this subchapter.

(C) The Administrator may conditionally register a pesticide containing an active ingredient not contained in any currently registered pesticide for a period reasonably sufficient for the generation and submission of required data (which are lacking because a period reasonably sufficient for generation of the data has not elapsed since the Administrator first imposed the data requirement) on the condition that by the end of such period the Administrator receives such data and the data do not meet or exceed risk criteria enumerated in regulations issued under this subchapter, and on such other conditions as the Administrator may prescribe. A conditional registration under this subparagraph shall be granted only if the Administrator determines that use of the pesticide during such period will not cause any unreasonable adverse effect on the environment, and that use of the pesticide is in the public interest.

(8) Interim administrative review

Notwithstanding any other provision of this subchapter, the Administrator may not initiate a public interim administrative review process to develop a risk-benefit evaluation of the ingredients of a pesticide or any of its uses prior to initiating a formal action to cancel, suspend, or deny registration of such pesticide, required under this subchapter, unless such interim administrative process is based on a validated test or other significant evidence raising prudent concerns of unreasonable adverse risk to man or to the environment. Notice of the definition of

the terms "validated test" and "other significant evidence" as used herein shall be published by the Administrator in the Federal Register.

(9) Labeling

(A) Additional statements

Subject to subparagraphs (B) and (C), it shall not be a violation of this subchapter for a registrant to modify the labeling of an antimicrobial pesticide product to include relevant information on product efficacy, product composition, container composition or design, or other characteristics that do not relate to any pesticidal claim or pesticidal activity.

(B) Requirements

Proposed labeling information under subparagraph (A) shall not be false or misleading, shall not conflict with or detract from any statement required by law or the Administrator as a condition of registration, and shall be substantiated on the request of the Administrator.

(C) Notification and disapproval

(i) Notification A registration may be modified under subparagraph (A) if—

(I) the registrant notifies the Administrator in writing not later than 60 days prior to distribution or sale of a product bearing the modified labeling; and

(II) the Administrator does not disapprove of the modification under clause (ii).

(ii) Disapproval Not later than 30 days after receipt of a notification under clause (i), the Administrator may disapprove the modification by sending the registrant notification in writing stating that the proposed language is not acceptable and stating the reasons why the Administrator finds the proposed modification unacceptable.

(iii) Restriction on sale A registrant may not sell or distribute a product bearing a disapproved modification.

(iv) Objection A registrant may file an objection in writing to a disapproval under clause (ii) not later than 30 days after receipt of notification of the disapproval.

(v) Final action A decision by the Administrator following receipt and consideration of an objection filed under clause (iv) shall be considered a

final agency action.

(D) Use dilution

The label or labeling required under this subchapter for an antimicrobial pesticide that is or may be diluted for use may have a different statement of caution or protective measures for use of the recommended diluted solution of the pesticide than for use of a concentrate of the pesticide if the Administrator determines that—

(i) adequate data have been submitted to support the statement proposed for the diluted solution uses; and

(ii) the label or labeling provides adequate protection for exposure to the diluted solution of the pesticide.

(10) Expedited registration of pesticides

(A) Not later than 1 year after August 3, 1996, the Administrator shall, utilizing public comment, develop procedures and guidelines, and expedite the review of an application for registration of a pesticide or an amendment to a registration that satisfies such guidelines.

(B) Any application for registration or an amendment, including biological and conventional pesticides, will be considered for expedited review under this paragraph. An application for registration or an amendment shall qualify for expedited review if use of the pesticide proposed by the application may reasonably be expected to accomplish 1 or more of the following:

(i) Reduce the risks of pesticides to human health.

(ii) Reduce the risks of pesticides to nontarget organisms.

(iii) Reduce the potential for contamination of groundwater, surface water, or other valued environmental resources.

(iv) Broaden the adoption of integrated pest management strategies, or make such strategies more available or more effective.

(C) The Administrator, not later than 30 days after receipt of an application for expedited review, shall notify the applicant whether the application is complete. If it is found to be incomplete, the Administrator may either reject the request for expedited review or ask the applicant for additional information to satisfy the guidelines developed under subparagraph (A).

(d) Classification of pesticides

(1) Classification for general use, restricted use, or both

(A) As a part of the registration of a pesticide the Administrator shall classify it as being for general use or for restricted use. If the Administrator determines that some of the uses for which the pesticide is registered should be for general use and that other uses for which it is registered should be for restricted use, the Administrator shall classify it for both general use and restricted use. Pesticide uses may be classified by regulation on the initial classification, and registered pesticides may be classified prior to reregistration. If some of the uses of the pesticide are classified for general use, and other uses are classified for restricted use, the directions relating to its general uses shall be clearly separated and distinguished from those directions relating to its restricted uses. The Administrator may require that its packaging and labeling for restricted uses shall be clearly distinguishable from its packaging and labeling for general uses.

(B) If the Administrator determines that the pesticide, when applied in accordance with its directions for use, warnings and cautions and for the uses for which it is registered, or for one or more of such uses, or in accordance with a widespread and commonly recognized practice, will not generally cause unreasonable adverse effects on the environment, the Administrator will classify the pesticide, or the particular use or uses of the pesticide to which the determination applies, for general use.

(C) If the Administrator determines that the pesticide, when applied in accordance with its directions for use, warnings and cautions and for the uses for which it is registered, or for one or more of such uses, or in accordance with a widespread and commonly recognized practice, may generally cause, without additional regulatory restrictions, unreasonable adverse effects on the environment, including injury to the applicator, the Administrator shall classify the pesticide, or the particular use or uses to which the determination applies, for restricted use:

(i) If the Administrator classifies a pesticide, or one or more uses of such pesticide, for restricted use because of a determination that the acute dermal or inhalation toxicity of the pesticide presents a hazard to the applicator or other persons, the pesticide shall be applied for any use to which the restricted classification applies only by or under the direct supervision of a certified applicator.

(ii) If the Administrator classifies a pesticide, or one or more uses of such pesticide, for restricted use because of a determination that its use without additional regulatory restriction may cause unreasonable adverse effects on the environment, the pesticide shall be applied for any use to which the determination applies only by or under the direct supervision of a certified

applicator, or subject to such other restrictions as the Administrator may provide by regulation. Any such regulation shall be reviewable in the appropriate court of appeals upon petition of a person adversely affected filed within 60 days of the publication of the regulation in final form.

(2) Change in classification

If the Administrator determines that a change in the classification of any use of a pesticide from general use to restricted use is necessary to prevent unreasonable adverse effects on the environment, the Administrator shall notify the registrant of such pesticide of such determination at least forty-five days before making the change and shall publish the proposed change in the Federal Register. The registrant, or other interested person with the concurrence of the registrant, may seek relief from such determination under section 136d (b) of this title.

(3) Change in classification from restricted use to general use

The registrant of any pesticide with one or more uses classified for restricted use may petition the Administrator to change any such classification from restricted to general use. Such petition shall set out the basis for the registrant's position that restricted use classification is unnecessary because classification of the pesticide for general use would not cause unreasonable adverse effects on the environment. The Administrator, within sixty days after receiving such petition, shall notify the registrant whether the petition has been granted or denied. Any denial shall contain an explanation therefor and any such denial shall be subject to judicial review under section 136n of this title.

(e) Products with same formulation and claims

Products which have the same formulation, are manufactured by the same person, the labeling of which contains the same claims, and the labels of which bear a designation identifying the product as the same pesticide may be registered as a single pesticide; and additional names and labels shall be added to the registration by supplemental statements.

(f) Miscellaneous

(1) Effect of change of labeling or formulation

If the labeling or formulation for a pesticide is changed, the registration shall be amended to reflect such change if the Administrator determines that the change will not violate any provision of this subchapter.

(2) Registration not a defense

In no event shall registration of an article be construed as a defense for the commission of any offense under this subchapter. As long as no cancellation proceedings are in effect registration of a pesticide shall be prima facie evidence that the pesticide, its labeling and packaging comply with the registration provisions of the subchapter.

(3) Authority to consult other Federal agencies

In connection with consideration of any registration or application for registration under this section, the Administrator may consult with any other Federal agency.

(4) Mixtures of nitrogen stabilizers and fertilizer products

Any mixture or other combination of—

(A) 1 or more nitrogen stabilizers registered under this subchapter; and

(B) 1 or more fertilizer products,

shall not be subject to the provisions of this section or sections 136a–1, 136c, 136e, 136m, and 136o (a)(2) of this title if the mixture or other combination is accompanied by the labeling required under this subchapter for the nitrogen stabilizer contained in the mixture or other combination, the mixture or combination is mixed or combined in accordance with such labeling, and the mixture or combination does not contain any active ingredient other than the nitrogen stabilizer.

(g) Registration review

(1) General rule

(A) Periodic review

The registrations of pesticides are to be periodically reviewed. The Administrator shall by regulation establish a procedure for accomplishing the periodic review of registrations. The goal of these regulations shall be a review of a pesticide's registration every 15 years. No registration shall be canceled as a result of the registration review process unless the Administrator follows the procedures and substantive requirements of section 136d of this title.

(B) Limitation

Nothing in this subsection shall prohibit the Administrator from undertaking any other review of a pesticide pursuant to this subchapter.

(2) Data

(A) Submission required

The Administrator shall use the authority in subsection (c)(2)(B) of this section to require the submission of data when such data are necessary for a registration review.

(B) Data submission, compensation, and exemption

For purposes of this subsection, the provisions of subsections (c)(1), (c)(2)(B), and (c)(2)(D) of this section shall be utilized for and be applicable to any data required for registration review.

(h) Registration requirements for antimicrobial pesticides

(1) Evaluation of process

To the maximum extent practicable consistent with the degrees of risk presented by an antimicrobial pesticide and the type of review appropriate to evaluate the risks, the Administrator shall identify and evaluate reforms to the antimicrobial registration process that would reduce review periods existing as of August 3, 1996, for antimicrobial pesticide product registration applications and applications for amended registration of antimicrobial pesticide products, including—

(A) new antimicrobial active ingredients;

(B) new antimicrobial end-use products;

(C) substantially similar or identical antimicrobial pesticides; and

(D) amendments to antimicrobial pesticide registrations.

(2) Review time period reduction goal

Each reform identified under paragraph (1) shall be designed to achieve the goal of reducing the review period following submission of a complete application, consistent with the degree of risk, to a period of not more than—

(A) 540 days for a new antimicrobial active ingredient pesticide registration;

(B) 270 days for a new antimicrobial use of a registered active ingredient;

(C) 120 days for any other new antimicrobial product;

(D) 90 days for a substantially similar or identical antimicrobial product;

(E) 90 days for an amendment to an antimicrobial registration that does not require scientific review of data; and

(F) 120 days for an amendment to an antimicrobial registration that requires scientific review of data and that is not otherwise described in this paragraph.

(3) Implementation

(A) Proposed rulemaking

(i) Issuance Not later than 270 days after August 3, 1996, the Administrator shall publish in the Federal Register proposed regulations to accelerate and improve the review of antimicrobial pesticide products designed to implement, to the extent practicable, the goals set forth in paragraph (2).

(ii) Requirements Proposed regulations issued under clause (i) shall—

(I) define the various classes of antimicrobial use patterns, including household, industrial, and institutional disinfectants and sanitizing pesticides, preservatives, water treatment, and pulp and paper mill additives, and other such products intended to disinfect, sanitize, reduce, or mitigate growth or development of microbiological organisms, or protect inanimate objects, industrial processes or systems, surfaces, water, or other chemical substances from contamination, fouling, or deterioration caused by bacteria, viruses, fungi, protozoa, algae, or slime;

(II) differentiate the types of review undertaken for antimicrobial pesticides;

(III) conform the degree and type of review to the risks and benefits presented by antimicrobial pesticides and the function of review under this subchapter, considering the use patterns of the product, toxicity, expected exposure, and product type;

(IV) ensure that the registration process is sufficient to maintain antimicrobial pesticide efficacy and that antimicrobial pesticide products continue to meet product performance standards and effectiveness levels for each type of label claim made; and

(V) implement effective and reliable deadlines for process management.

(iii) Comments In developing the proposed regulations, the Administrator shall solicit the views from registrants and other affected parties to maximize the effectiveness of the rule development process.

(B) Final regulations

(i) Issuance The Administrator shall issue final regulations not later than 240 days after the close of the comment period for the proposed regulations.

(ii) Failure to meet goal If a goal described in paragraph (2) is not met by the final regulations, the Administrator shall identify the goal, explain why the goal was not attained, describe the element of the regulations included instead, and identify future steps to attain the goal.

(iii) Requirements In issuing final regulations, the Administrator shall—

(I) consider the establishment of a certification process for regulatory actions involving risks that can be responsibly managed, consistent with the degree of risk, in the most cost-efficient manner;

(II) consider the establishment of a certification process by approved laboratories as an adjunct to the review process;

(III) use all appropriate and cost-effective review mechanisms, including—

(aa) expanded use of notification and non-notification procedures;

(bb) revised procedures for application review; and

(cc) allocation of appropriate resources to ensure streamlined management of antimicrobial pesticide registrations; and

(IV) clarify criteria for determination of the completeness of an application.

(C) Expedited review

This subsection does not affect the requirements or extend the deadlines or review periods contained in subsection (c)(3) of this section.

(D) Alternative review periods

If the final regulations to carry out this paragraph are not effective 630 days after August 3, 1996, until the final regulations become effective, the review period, beginning on the date of receipt by the Agency of a complete

application, shall be—

(i) 2 years for a new antimicrobial active ingredient pesticide registration;

(ii) 1 year for a new antimicrobial use of a registered active ingredient;

(iii) 180 days for any other new antimicrobial product;

(iv) 90 days for a substantially similar or identical antimicrobial product;

(v) 90 days for an amendment to an antimicrobial registration that does not require scientific review of data; and

(vi) 120 days for an amendment to an antimicrobial registration that requires scientific review of data and that is not otherwise described in this subparagraph.

(E) Wood preservatives

An application for the registration, or for an amendment to the registration, of a wood preservative product for which a claim of pesticidal activity listed in section 136 (mm) of this title is made (regardless of any other pesticidal claim that is made with respect to the product) shall be reviewed by the Administrator within the same period as that established under this paragraph for an antimicrobial pesticide product application, consistent with the degree of risk posed by the use of the wood preservative product, if the application requires the applicant to satisfy the same data requirements as are required to support an application for a wood preservative product that is an antimicrobial pesticide.

(F) Notification

(i) In general Subject to clause (iii), the Administrator shall notify an applicant whether an application has been granted or denied not later than the final day of the appropriate review period under this paragraph, unless the applicant and the Administrator agree to a later date.

(ii) Final decision If the Administrator fails to notify an applicant within the period of time required under clause (i), the failure shall be considered an agency action unlawfully withheld or unreasonably delayed for purposes of judicial review under chapter 7 of title 5.

(iii) Exemption This subparagraph does not apply to an application for an antimicrobial pesticide that is filed under subsection (c)(3)(B) of this section prior to 90 days after August 3, 1996.

(iv) Limitation Notwithstanding clause (ii), the failure of the Administrator to notify an applicant for an amendment to a registration for an antimicrobial pesticide shall not be judicially reviewable in a Federal or State court if the amendment requires scientific review of data within—

(I) the time period specified in subparagraph (D)(vi), in the absence of a final regulation under subparagraph (B); or

(II) the time period specified in paragraph (2)(F), if adopted in a final regulation under subparagraph (B).

(4) Annual report

(A) *Submission*

Beginning on August 3, 1996, and ending on the date that the goals under paragraph (2) are achieved, the Administrator shall, not later than March 1 of each year, prepare and submit an annual report to the Committee on Agriculture of the House of Representatives and the Committee on Agriculture, Nutrition, and Forestry of the Senate.

(B) Requirements

A report submitted under subparagraph (A) shall include a description of—

(i) measures taken to reduce the backlog of pending registration applications;

(ii) progress toward achieving reforms under this subsection; and

(iii) recommendations to improve the activities of the Agency pertaining to antimicrobial registrations.

§ 136. Definitions

How Current is This?

For purposes of this subchapter—

(a) Active ingredient

The term "active ingredient" means—

(1) in the case of a pesticide other than a plant regulator, defoliant, desiccant, or nitrogen stabilizer, an ingredient which will prevent, destroy, repel, or mitigate any pest;

(2) in the case of a plant regulator, an ingredient which, through physiological action, will accelerate or retard the rate of growth or rate of maturation or otherwise alter the behavior of ornamental or crop plants or the product thereof;

(3) in the case of a defoliant, an ingredient which will cause the leaves or foliage to drop from a plant;

(4) in the case of a desiccant, an ingredient which will artificially accelerate the drying of plant tissue; and

(5) in the case of a nitrogen stabilizer, an ingredient which will prevent or hinder the process of nitrification, denitrification, ammonia volatilization, or urease production through action affecting soil bacteria.

(b) Administrator

The term "Administrator" means the Administrator of the Environmental Protection Agency.

(c) Adulterated

The term "adulterated" applies to any pesticide if—

(1) its strength or purity falls below the professed standard of quality as expressed on its labeling under which it is sold;

(2) any substance has been substituted wholly or in part for the pesticide; or

(3) any valuable constituent of the pesticide has been wholly or in part abstracted.

(d) Animal

The term "animal" means all vertebrate and invertebrate species, including but not limited to man and other mammals, birds, fish, and shellfish.

(e) Certified applicator, etc.

(1) Certified applicator

The term "certified applicator" means any individual who is certified under section 136i of this title as authorized to use or supervise the use of any pesticide which is classified for restricted use. Any applicator who holds or applies registered pesticides, or uses dilutions of registered pesticides consistent with subsection (ee) of this section, only to provide a service of controlling pests without delivering any unapplied pesticide to any person so served is not deemed to be a seller or distributor of pesticides under this subchapter.

(2) Private applicator

The term "private applicator" means a certified applicator who uses or supervises the use of any pesticide which is classified for restricted use for purposes of producing any agricultural commodity on property owned or rented by the applicator or the applicator's employer or (if applied without compensation other than trading of personal services between producers of agricultural commodities) on the property of another person.

(3) Commercial applicator

The term "commercial applicator" means an applicator (whether or not the applicator is a private applicator with respect to some uses) who uses or supervises the use of any pesticide which is classified for restricted use for any purpose or on any property other than as provided by paragraph (2).

(4) Under the direct supervision of a certified applicator

Unless otherwise prescribed by its labeling, a pesticide shall be considered to be applied under the direct supervision of a certified applicator if it is applied by a competent person acting under the instructions and control of a certified applicator who is available if and when needed, even though such certified applicator is not physically present at the time and place the pesticide is applied.

(f) Defoliant

The term "defoliant" means any substance of mixture of substances intended for causing the leaves or foliage to drop from a plant, with or without causing abscission.

(g) Desiccant

The term "desiccant" means any substance or mixture of substances intended for artificially accelerating the drying of plant tissue.

(h) Device

The term "device" means any instrument or contrivance (other than a firearm) which is intended for trapping, destroying, repelling, or mitigating any pest or any other form of plant or animal life (other than man and other than bacteria, virus, or other microorganism on or in living man or other living animals); but not including equipment used for the application of pesticides when sold separately therefrom.

(i) District court

The term "district court" means a United States district court, the District Court of Guam, the District Court of the Virgin Islands, and the highest court of American Samoa.

(j) Environment

The term "environment" includes water, air, land, and all plants and man and other animals living therein, and the interrelationships which exist among these.

(k) Fungus

The term "fungus" means any non-chlorophyll-bearing thallophyte (that is, any non-chlorophyll-bearing plant of a lower order than mosses and liverworts), as for example, rust, smut, mildew, mold, yeast, and bacteria, except those on or in living man or other animals and those on or in processed food, beverages, or pharmaceuticals.

(l) Imminent hazard

The term "imminent hazard" means a situation which exists when the continued use of a pesticide during the time required for cancellation proceeding would be likely to result in

unreasonable adverse effects on the environment or will involve unreasonable hazard to the survival of a species declared endangered or threatened by the Secretary pursuant to the Endangered Species Act of 1973 [16 U.S.C. 1531 et seq.].

(m) Inert ingredient

The term "inert ingredient" means an ingredient which is not active.

(n) Ingredient statement

The term "ingredient statement" means a statement which contains—

(1) the name and percentage of each active ingredient, and the total percentage of all inert ingredients, in the pesticide; and

(2) if the pesticide contains arsenic in any form, a statement of the percentages of total and water soluble arsenic, calculated as elementary arsenic.

(o) Insect

The term "insect" means any of the numerous small invertebrate animals generally having the body more or less obviously segmented, for the most part belonging to the class insecta, comprising six-legged, usually winged forms, as for example, beetles, bugs, bees, flies, and to other allied classes of anthropods whose members are wingless and usually have more than six legs, as for example, spiders, mites, ticks, centipedes, and wood lice.

(p) Label and labeling

(1) Label

The term "label" means the written, printed, or graphic matter on, or attached to, the pesticide or device or any of its containers or wrappers.

(2) Labeling

The term "labeling" means all labels and all other written, printed, or graphic matter—

(A) accompanying the pesticide or device at any time; or

(B) to which reference is made on the label or in literature accompanying the

pesticide or device, except to current official publications of the Environmental Protection Agency, the United States Departments of Agriculture and Interior, the Department of Health and Human Services, State experiment stations, State agricultural colleges, and other similar Federal or State institutions or agencies authorized by law to conduct research in the field of pesticides.

(q) Misbranded

(1) A pesticide is misbranded if—

(A) its labeling bears any statement, design, or graphic representation relative thereto or to its ingredients which is false or misleading in any particular;

(B) it is contained in a package or other container or wrapping which does not conform to the standards established by the Administrator pursuant to section 136w (c)(3) of this title;

(C) it is an imitation of, or is offered for sale under the name of, another pesticide;

(D) its label does not bear the registration number assigned under section 136e of this title to each establishment in which it was produced;

(E) any word, statement, or other information required by or under authority of this subchapter to appear on the label or labeling is not prominently placed thereon with such conspicuousness (as compared with other words, statements, designs, or graphic matter in the labeling) and in such terms as to render it likely to be read and understood by the ordinary individual under customary conditions of purchase and use;

(F) the labeling accompanying it does not contain directions for use which are necessary for effecting the purpose for which the product is intended and if complied with, together with any requirements imposed under section 136a (d) of this title, are adequate to protect health and the environment;

(G) the label does not contain a warning or caution statement which may be necessary and if complied with, together with any requirements imposed under section 136a (d) of this title, is adequate to protect health and the environment; or

(H) in the case of a pesticide not registered in accordance with section 136a of this title and intended for export, the label does not contain, in words prominently placed thereon with such conspicuousness (as compared with other words, statements, designs, or graphic matter in the labeling) as to render it likely to be noted by the ordinary individual under customary conditions of purchase and use, the following: "Not Registered for Use in the United States of America".

(2) A pesticide is misbranded if—

(A) the label does not bear an ingredient statement on that part of the immediate container (and on the outside container or wrapper of the retail package, if there be one, through which the ingredient statement on the immediate container cannot be clearly read) which is presented or displayed under customary conditions or purchase, except that a pesticide is not misbranded under this subparagraph if—

(i) The size or form of the immediate container, or the outside container or wrapper of the retail package, makes it impracticable to place the ingredient statement on the part which is presented or displayed under customary conditions of purchase; and

(ii) the ingredient statement appears prominently on another part of the immediate container, or outside container or wrapper, permitted by the Administrator;

(B) the labeling does not contain a statement of the use classification under which the product is registered;

(C) there is not affixed to its container, and to the outside container or wrapper of the retail package, if there be one, through which the required information on the immediate container cannot be clearly read, a label bearing—

(i) the name and address of the producer, registrant, or person for whom produced;

(ii) the name, brand, or trademark under which the pesticide is sold;

(iii) the net weight or measure of the content, except that the Administrator may permit reasonable variations; and

(iv) when required by regulation of the Administrator to effectuate the purposes of this subchapter, the registration number assigned to the pesticide under this subchapter, and the use classification; and

(D) the pesticide contains any substance or substances in quantities highly toxic to man, unless the label shall bear, in addition to any other matter required by this subchapter—

(i) the skull and crossbones;

(ii) the word "poison" prominently in red on a background of distinctly contrasting color; and

(iii) a statement of a practical treatment (first aid or otherwise) in case of poisoning by the pesticide.

(r) Nematode

The term "nematode" means invertebrate animals of the phylum nemathelminthes and class nematoda, that is, unsegmented round worms with elongated, fusiform, or saclike bodies covered with cuticle, and inhabiting soil, water, plants, or plant parts; may also be called nemas or eelworms.

(s) Person

The term "person" means any individual, partnership, association, corporation, or any organized group of persons whether incorporated or not.

(t) Pest

The term "pest" means

(1) any insect, rodent, nematode, fungus, weed, or

(2) any other form of terrestrial or aquatic plant or animal life or virus, bacteria, or other micro-organism (except viruses, bacteria, or other micro-organisms on or in living man or other living animals) which the Administrator declares to be a pest under section 136w (c)(1) of this title.

(u) Pesticide

The term "pesticide" means

(1) any substance or mixture of substances intended for preventing, destroying, repelling, or mitigating any pest,

(2) any substance or mixture of substances intended for use as a plant regulator, defoliant, or desiccant, and

(3) any nitrogen stabilizer, except that the term "pesticide" shall not include any article that is a "new animal drug" within the meaning of section 321 (w) [1] of title 21, that has been determined by the Secretary of Health and Human Services not to be a new animal drug by a regulation establishing conditions of use for the article, or that is an

animal feed within the meaning of section 321 (x) [1] of title 21 bearing or containing a new animal drug. The term "pesticide" does not include liquid chemical sterilant products (including any sterilant or subordinate disinfectant claims on such products) for use on a critical or semi-critical device, as defined in section 321 of title 21. For purposes of the preceding sentence, the term "critical device" includes any device which is introduced directly into the human body, either into or in contact with the bloodstream or normally sterile areas of the body and the term "semi-critical device" includes any device which contacts intact mucous membranes but which does not ordinarily penetrate the blood barrier or otherwise enter normally sterile areas of the body.

(v) Plant regulator

The term "plant regulator" means 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, but shall not include substances to the extent that they are intended as plant nutrients, trace elements, nutritional chemicals, plant inoculants, and soil amendments. Also, the term "plant regulator" shall not be required to include any of such of those nutrient mixtures or soil amendments as are commonly known as vitamin-hormone horticultural products, intended for improvement, maintenance, survival, health, and propagation of plants, and as are not for pest destruction and are nontoxic, nonpoisonous in the undiluted packaged concentration.

(w) Producer and produce

The term "producer" means the person who manufactures, prepares, compounds, propagates, or processes any pesticide or device or active ingredient used in producing a pesticide. The term "produce" means to manufacture, prepare, compound, propagate, or process any pesticide or device or active ingredient used in producing a pesticide. The dilution by individuals of formulated pesticides for their own use and according to the directions on registered labels shall not of itself result in such individuals being included in the definition of "producer" for the purposes of this subchapter.

(x) Protect health and the environment

The terms "protect health and the environment" and "protection of health and the environment" mean protection against any unreasonable adverse effects on the environment.

(y) Registrant

The term "registrant" means a person who has registered any pesticide pursuant to the provisions of this subchapter.

(z) Registration

The term "registration" includes reregistration.

(aa) State

The term "State" means a State, the District of Columbia, the Commonwealth of Puerto Rico, the Virgin Islands, Guam, the Trust Territory of the Pacific Islands, and American Samoa.

(bb) Unreasonable adverse effects on the environment

The term "unreasonable adverse effects on the environment" means

(1) any unreasonable risk to man or the environment, taking into account the economic, social, and environmental costs and benefits of the use of any pesticide, or

(2) a human dietary risk from residues that result from a use of a pesticide in or on any food inconsistent with the standard under section 346a of title 21. The Administrator shall consider the risks and benefits of public health pesticides separate from the risks and benefits of other pesticides. In weighing any regulatory action concerning a public health pesticide under this subchapter, the Administrator shall weigh any risks of the pesticide against the health risks such as the diseases transmitted by the vector to be controlled by the pesticide.

(cc) Weed

The term "weed" means any plant which grows where not wanted.

(dd) Establishment

The term "establishment" means any place where a pesticide or device or active ingredient used in producing a pesticide is produced, or held, for distribution or sale.

(ee) To use any registered pesticide in a manner inconsistent with its labeling

The term "to use any registered pesticide in a manner inconsistent with its labeling" means to use any registered pesticide in a manner not permitted by the

labeling, except that the term shall not include

(1) applying a pesticide at any dosage, concentration, or frequency less than that specified on the labeling unless the labeling specifically prohibits deviation from the specified dosage, concentration, or frequency,

(2) applying a pesticide against any target pest not specified on the labeling if the application is to the crop, animal, or site specified on the labeling, unless the Administrator has required that the labeling specifically state that the pesticide may be used only for the pests specified on the labeling after the Administrator has determined that the use of the pesticide against other pests would cause an unreasonable adverse effect on the environment,

(3) employing any method of application not prohibited by the labeling unless the labeling specifically states that the product may be applied only by the methods specified on the labeling,

(4) mixing a pesticide or pesticides with a fertilizer when such mixture is not prohibited by the labeling,

(5) any use of a pesticide in conformance with section 136c, 136p, or 136v of this title, or

(6) any use of a pesticide in a manner that the Administrator determines to be consistent with the purposes of this subchapter. After March 31, 1979, the term shall not include the use of a pesticide for agricultural or forestry purposes at a dilution less than label dosage unless before or after that date the Administrator issues a regulation or advisory opinion consistent with the study provided for in section 27(b) of the Federal Pesticide Act of 1978, which regulation or advisory opinion specifically requires the use of definite amounts of dilution.

(ff) Outstanding data requirement

(1) In general

The term "outstanding data requirement" means a requirement for any study, information, or data that is necessary to make a determination under section 136a (c)(5) of this title and which study, information, or data—

(A) has not been submitted to the Administrator; or

(B) if submitted to the Administrator, the Administrator has determined must be resubmitted because it is not valid, complete, or adequate to make a determination under section 136a (c)(5) of this title and the regulations and guidelines issued under such section.

(2) Factors

In making a determination under paragraph (1)(B) respecting a study, the Administrator shall examine, at a minimum, relevant protocols, documentation of the conduct and analysis of the study, and the results of the study to determine whether the study and the results of the study fulfill the data requirement for which the study was submitted to the Administrator.

(gg) To distribute or sell

The term "to distribute or sell" means to distribute, sell, offer for sale, hold for distribution, hold for sale, hold for shipment, ship, deliver for shipment, release for shipment, or receive and (having so received) deliver or offer to deliver. The term does not include the holding or application of registered pesticides or use dilutions thereof by any applicator who provides a service of controlling pests without delivering any unapplied pesticide to any person so served.

(hh) Nitrogen stabilizer

The term "nitrogen stabilizer" means any substance or mixture of substances intended for preventing or hindering the process of nitrification, denitrification, ammonia volatilization, or urease production through action upon soil bacteria. Such term shall not include—

(1) dicyandiamide;

(2) ammonium thiosulfate; or

(3) any•substance•or•mixture•of•substances.— [2]

(A) that was not registered pursuant to section 136a of this title prior to January 1, 1992; and

(B) that was in commercial agronomic use prior to January 1, 1992, with respect to which after January 1, 1992, the distributor or seller of the substance or mixture has made no specific claim of prevention or hindering of the process of nitrification, denitrification, ammonia volatilization [3] urease production regardless of the actual use or purpose for, or future use or purpose for, the substance or mixture.

Statements made in materials required to be submitted to any State legislative or regulatory authority, or required by such authority to be included in the labeling or other literature accompanying any such substance or mixture shall not be deemed a specific claim within the meaning of this subsection.

(jj) •4 Maintenance applicator

The term "maintenance applicator" means any individual who, in the principal course of such individual's employment, uses, or supervises the use of, a pesticide not classified for restricted use (other than a ready to use consumer products pesticide); for the purpose of providing structural pest control or lawn pest control including janitors, general maintenance personnel, sanitation personnel, and grounds maintenance personnel. The term "maintenance applicator" does not include private applicators as defined in subsection (e)(2) of this section; individuals who use antimicrobial pesticides, sanitizers or disinfectants; individuals employed by Federal, State, and local governments or any political subdivisions thereof, or individuals who use pesticides not classified for restricted use in or around their homes, boats, sod farms, nurseries, greenhouses, or other noncommercial property.

(kk) Service technician

The term "service technician" means any individual who uses or supervises the use of pesticides (other than a ready to use consumer products pesticide) for the purpose of providing structural pest control or lawn pest control on the property of another for a fee. The term "service technician" does not include individuals who use antimicrobial pesticides, sanitizers or disinfectants; or who otherwise apply ready to use consumer products pesticides.

(ll) Minor use

The term "minor use" means the use of a pesticide on an animal, on a commercial agricultural crop or site, or for the protection of public health where—

(1) the total United States acreage for the crop is less than 300,000 acres, as determined by the Secretary of Agriculture; or

(2) the Administrator, in consultation with the Secretary of Agriculture, determines that, based on information provided by an applicant for registration or a registrant, the use does not provide sufficient economic incentive to support the initial registration or continuing registration of a pesticide for such use and—

(A) there are insufficient efficacious alternative registered pesticides available for the use;

(B) the alternatives to the pesticide use pose greater risks to the environment or human health;

(C) the minor use pesticide plays or will play a significant part in managing pest

resistance; or

(D) the minor use pesticide plays or will play a significant part in an integrated pest management program.

The status as a minor use under this subsection shall continue as long as the Administrator has not determined that, based on existing data, such use may cause an unreasonable adverse effect on the environment and the use otherwise qualifies for *such status*.

(mm) Antimicrobial pesticide

(1) In general

The term "antimicrobial pesticide" means a pesticide that—

(A) is intended to—

(i) disinfect, sanitize, reduce, or mitigate growth or development of microbiological organisms; or

(ii) protect inanimate objects, industrial processes or systems, surfaces, water, or other chemical substances from contamination, fouling, or deterioration caused by bacteria, viruses, fungi, protozoa, algae, or slime; and

(B) in the intended use is exempt from, or otherwise not subject to, a tolerance under section 346a of title 21 or a food additive regulation under section 348 of title 21.

(2) Excluded products

The term "antimicrobial pesticide" does not include—

(A) a wood preservative or antifouling paint product for which a claim of pesticidal activity other than or in addition to an activity described in paragraph (1) is made;

(B) an agricultural fungicide product; or

(C) an aquatic herbicide product.

(3) Included products

The term "antimicrobial pesticide" does include any other chemical sterilant product (other than liquid chemical sterilant products exempt under subsection (u)

of this section), any other disinfectant product, any other industrial microbiocide product, and any other preservative product that is not excluded by paragraph (2).

(nn) Public health pesticide

The term "public health pesticide" means any minor use pesticide product registered for use and used predominantly in public health programs for vector control or for other recognized health protection uses, including the prevention or mitigation of viruses, bacteria, or other microorganisms (other than viruses, bacteria, or other microorganisms on or in living man or other living animal) that pose a threat to public health.

(oo) Vector

The term "vector" means any organism capable of transmitting the causative agent of human disease or capable of producing human discomfort or injury, including mosquitoes, flies, fleas, cockroaches, or other insects and ticks, mites, or rats.

Indole-3-Butyric Acid (046701) Fact Sheet

Related Information

- Regulating Biopesticides
- Active Ingredient Index

Information related to this page:

- Factsheet
- Federal Register Notices
- Products
- Registrants
- Technical Document (PDF)
  (60 pp, 1.1 MB about PDF)

Issued: 8/1/00

On This Page

Summary

Indole-3-butyric acid enhances the growth and development of food crops and ornamentals when applied to soil, cuttings, or leaves. Because it is similar in structure to naturally occurring substances and is used in tiny amounts, this plant growth regulator poses no known risks to humans or the environment.

I. Description of the Active Ingredient

Indole-3-butyric acid is a substance that is closely related in structure and function to a natural growth regulator found in plants. Indole-3-butyric acid is used on many crops and ornamentals to promote growth and development of roots, flowers and fruits, and to increase crop yields. Growers find it more effective and efficient than its natural counterpart because plants cannot break it down as quickly. No harm to humans or the environment is expected to result from use of indole-3-butyric acid.

II. Use Sites, Target Pests, and Application Methods

- **Use Sites:** Many food and feed crops; ornamental turf and nursery plants
- **Uses:** Growth enhancer to increase both yield and quality.
- **Application Methods:** Applied to soil or plants as spray. Also used as a dip for cuttings.

III. Assessing Risks to Human Health

With the exception of certain workers, no harm is expected from use of indole-3-butyric acid. The active ingredient is not toxic to humans or other mammals. Furthermore, indole-3-butyric acid is effective at very low concentrations--often several orders of magnitude below 1%. It is applied at very low rates compared with most other pesticides. In animals, indole-3-butyric acid is rapidly broken down to a closely related, harmless chemical that occurs naturally in living organisms.

Eye irritation to certain workers is EPA' s only health concern for products containing indole-3-butyric acid. For products that may cause eye irritation, workers (such as mixers and applicators) are required to use protective eyewear, such as goggles, face shield, or safety glasses.

IV. Assessing Risks to the Environment

No risks to the environment are expected from use of this active ingredient because 1) it does not harm animals or plants in the tiny amounts used, 2) it acts as a plant growth enhancer, 3) it does not persist in the environment, 4) it is closely related to naturally occurring substances.

V. Regulatory Information

Products containing indole-3-butyric acid were initially registered (licensed for sale and distribution) in 1960 for use on ornamental plant cuttings and transplants. As part of EPA' s ongoing review to ensure that pesticide products meet current standards, the chemical was reviewed and found eligible for reregistration in 1992. As of May 2000, there were more than 40 products containing indole-3-butyric acid as an active ingredient.

VI. Registrant Information

Many companies have registered pesticide products that contain indole-3-butyric acid.

VII. Additional Contact Information

Ombudsman, Biopesticides and Pollution Prevention Division (7511P)
Office of Pesticide Programs
Environmental Protection Agency
1200 Pennsylvania Avenue, NW
Washington, D.C. 20460

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Rita Kumar

United States Environmental Protection Agency
Office of Pesticide Programs
August 1992

EPA

Reregistration Eligibility Document (RED)

Indole-3-Butyric Acid

REREGISTRATION ELIGIBILITY DOCUMENT

INDOLE-3-BUTYRIC ACID

LIST B

CASE 2330

ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF PESTICIDE PROGRAMS
SPECIAL REVIEW AND REREGISTRATION DIVISION
WASHINGTON, D.C.

INDOLE-3-BUTYRIC ACID REREGISTRATION ELIGIBILITY TEAM

Office of Pesticide Programs:

Special Review and Reregistration Division

Ruby Whiters	Accelerated Reregistration Branch
Jay Ellenberger	Accelerated Reregistration Branch

Health Effects Division

Thomas McClintock	Science Analysis Branch
Jeff Evans	Occupational and Residential Exposure Branch

Environmental Fate and Effects Division

Les Touart	Ecological Effects Branch
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Skee Jones	Environmental Fate and Groundwater Branch

Registration Division

Cynthia Giles-Parker	Fungicide and Herbicide Branch
Sangeeta Khattar	Fungicide and Herbicide Branch
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Biological and Economic Analysis Branch

Neil Anderson	Biological Analysis Branch
Gabe Patrick	Biological Analysis Branch

Policy and Special Projects Staff

Kennan Garvey

Office of General Counsel

Eran Gasko

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Beverly Updike

GLOSSARY OF TERMS AND ABBREVIATIONS

CAS	Chemical Abstracts Service
EPA	U.S. Environmental Protection Agency
FIFRA	Federal Insecticide, Fungicide and Rodenticide Act
MRID	Master Record Identification (number)
	EPA's system of recording and tracking studies submitted.
RED	Reregistration Eligibility Document

TABLE OF CONTENTS

EXECUTIVE SUMMARY

Pesticide products containing indole-3-butyric acid (IBA) as the sole active ingredient, or in combination with other active ingredients, have been registered since October 1960. These products have been registered for use on plant cuttings and transplants of nonfood, ornamental nursery stock to promote root growth and to reduce transplanting shock. In 1990, new products were registered for use on fruit and vegetable crops, field crops and ornamental turf to promote growth development of flowers and fruit and to increase crop yields. Thirty-one products are currently registered with the Environmental Protection Agency ("the Agency").

The Agency has assessed the available scientific information about this compound in relation to all its registered uses to determine its eligibility for reregistration. The data base for IBA is sufficient to allow the Agency to conduct a tentative risk assessment for all uses. Therefore, the Agency has determined that the products containing IBA for all uses are eligible for reregistration.

Before reregistering each product, the Agency is requiring confirmatory acute ecotoxicity data on the active ingredient, product specific data, and revised product labeling to be submitted within eight months from the issuance of this document. In an effort to reduce the time, resources, and number of animals needed to fulfill the acute toxicity data requirements for reregistration of end-use products containing IBA, the Agency has batched products which can be considered similar for purposes of acute toxicity. After reviewing these data and revised labels, the Agency will determine whether or not the conditions of the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) section 3(c)(5) have been met, that is, whether product composition and labeling are acceptable and the product's uses will not cause unreasonable adverse effects to humans or the environment. If these conditions are met, the Agency will reregister the products. Those products which contain other active ingredients will be eligible for reregistration only when the other active ingredients are determined to be eligible for reregistration.

I. INTRODUCTION

In 1988, FIFRA was amended to accelerate the reregistration of products with active ingredients registered prior to November 1, 1984. The amended Act provides a schedule for the reregistration process to be completed in nine years. There are five phases to the reregistration process. The first four phases of the process focus on identification of data requirements to support the reregistration of an active ingredient and the generation and submission of data to fulfill the requirements. The fifth phase is a review by the Agency of all data submitted to support reregistration.

Section 4(g)(2)(A) of FIFRA states that in Phase 5 "the Administrator shall determine whether pesticides containing such active ingredient are eligible for reregistration" before calling in data on products under section 4(g)(2)(B), and either reregistering products or taking "other appropriate regulatory action," under section 4(g)(2)(C) and (D). Thus, reregistration involves a thorough review of the scientific data base underlying a pesticide's registration. The purpose of the Agency's review is to reassess the potential hazards arising from the currently registered uses of the pesticide; to determine the need for additional data on health and environmental effects; and to determine whether the pesticide meets the "no unreasonable adverse effects" criterion of FIFRA section 3(c)(5).

This document presents the Agency's decision regarding the reregistration eligibility of indole-3-butyric acid (IBA). This document consists of five sections. Section I is this introduction. Section II describes IBA, its uses and regulatory history. Section III discusses the human health and environmental assessment based on the data available to the Agency. Section IV discusses the reregistration decision for IBA and Section V discusses product reregistration. Additional details concerning the Agency's review of available data are available on request.[1]

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[1] EPA's reviews of specific reports and information on the set of registered uses considered for EPA's analyses may be obtained from: EPA, Freedom of Information, 401, M St., S.W., Washington, D.C. 20460.

II. CASE OVERVIEW

A. Chemical Overview

The following active ingredient is covered by this Reregistration Eligibility Document.

Common Name: IBA

Chemical Name: Indole-3-Butyric Acid

CAS Registry Number: 133-32-4

Office of Pesticide Programs Chemical Code: 046701

Empirical Formula: $C_{12}H_{13}NO_2$

Trade and Other Names: Hormodin, Seradix

Basic Manufacturer: Syntex S.A., Chemical Division

B. Use Profile

The following is information on the current registered uses and application methods. A detailed table of all uses of indole-3-butyric acid is in Appendix A.

Type of Pesticide: biochemical pesticide, plant growth regulator

Use Sites: Greenhouse Nonfood - ornamental plants, shade trees and shrubs

Terrestrial Nonfood - ornamental plants, shade trees, shrubs, turf, sod, lawn, golf courses

Residential Indoor/Outdoor - ornamental plants, shade trees and shrubs

Terrestrial Food - fruit, vegetable, field crops

Formulation Types

Technical Grade: 97.0%
Formulations: Dust: 0.01% - 4.5%
Soluble Conc./Liquid: 0.0004% - 1.03%
Wettable Powder/Dust: 0.1 - 0.8%

Method of Application:

Powder (Dust): plant cuttings are dipped in dust or inserting the cuttings into rooting media.

Dust is blown on the cutting ends by means of a duster or blower.

Liquid: cuttings are dipped in solution prior to insertion into rooting media.

Solution is poured into planting hole pre-transplant and during transplant.

Immediately after laying turf, solution is broadcast over turf and then watered in after application.

Foliage is sprayed with solution to point of run-off.

Broadcast: application is made depending on the crop (either by ground or air equipment) at -- leaf stage; pinhead; first or early bloom; initial pegging; first sign of running; or after transplant; pre and post emergence.

Solution is applied through sprinkler including center pivot, lateral move, end tow, side roll, traveler, big gun, solid set, or hand move irrigation systems.

C. Regulatory History

As stated in the Executive Summary, products containing IBA were first registered in October 1960. IBA was originally registered for use on a variety of nonfood ornamental plants, shrubs and shade trees to promote and accelerate root formation of plant clippings and to reduce transplant shock. On October 1990, additional uses were registered for IBA which included fruit and vegetable crops, field crops, and ornamental turf.

On June 28, 1988, the Agency issued a Data Call-In Notice for data on pesticide products containing IBA as the active ingredient. The registrants responded by requesting a low volume minor use data waiver for all applicable guidelines. The Agency was later asked to classify IBA as a biochemical pesticide. Following review, the Agency designated IBA as a biochemical pesticide based on the following scientific reason: 1) IBA is similar in structure and functional identical to a naturally occurring plant hormone or auxin, indole-3-acetic acid.

III. SCIENCE ASSESSMENT OF INDOLE-3-BUTYRIC ACID

A. Product Chemistry Assessment

Indole-3-butyric acid is a synthetic plant hormone which structurally resembles 3-indole acetic acid (IAA), the primary growth hormone, naturally occurring in plants. Several naturally occurring plant hormones have been identified which display structural and physiological activity similar to this compound. Such plant compounds, which are intermediate metabolites produced during the synthesis of 3-indole acetic acid from tryptophan, are thought to be converted to IAA prior to being considered effective when applied to auxin deficient plant tissue.

The molecular weight of IBA is 203.23. IBA is odorless, white or slightly yellow crystals, and has a melting point of 123-125°C. IBA is practically insoluble in water and chloroform but soluble in alcohol, esters and acetone.[2] All generic chemistry data requirements for IBA have been satisfied. Appendix B and C includes references of these data.

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[2] The Merck Index, Eighth Edition p. 565.

B. Human Health Assessment

1. Toxicology Data

The Agency has waived all data requirements on the active ingredient because of the expected, extremely low exposures to those involved in the use of products containing IBA and due to the negligible dietary exposures expected from the use of IBA on food and feed crops. IBA is exempt from tolerances of residues on crops (40 CFR 180.1099). All registered products are formulated with IBA in low percentages of IBA, from 0.0004 to 4.5, and are applied in ultra-low quantities, up to 7 mg active ingredient/acre/crop season, for the crop uses. Use of products for the ornamental plant propagation use also results in low applicator exposure to IBA.

Additionally, these products have low acute toxicity as suggested from data of at least one formulated product (Toxicity Categories III and IV, no dermal irritation, and moderate eye irritation, perhaps from another active or an inert ingredient in the product formulation.[3] As discussed in other sections of this document, the Agency is requiring registrants to submit new, or reference, existing acute toxicology and chemistry studies for each product.

2. Occupational and Residential Exposure

There is potential for occupational exposure to IBA during dipping and transplanting activities and mixing, loading and spraying activities. However, since the Agency does not have concerns about any toxicological endpoints, the Agency has not required exposure data. The Agency has no significant exposure concerns other than appropriate label precautions for eye protection for mixers, loaders, and applicators. For pesticide products in Toxicity Category II for primary eye irritation, the following protective eyewear are required: goggles; face shield; or safety with glasses with front, brow, and temple protection.

[3] MRID #s 41661402, 41661403, 41661404, 41661406

3. Human Risk Assessment

As discussed above, the potential risks to humans from occupational exposure to IBA are considered negligible due to: a) the lack of toxicological concerns, b) the low volume/minor use of the product, and c) IBA's structural resemblance to naturally occurring plant hormones.

C. Environmental Assessment

1. Ecological Effects and Environmental Fate Data

Although data from environmental fate and ecological effects studies have not been submitted, the Agency believes that there is sufficient information available to tentatively assess potential environmental risks resulting from the current uses of IBA. Accordingly, the Agency at this time will require environmental effects (ecotoxicity) data only for confirmatory purposes.

The terrestrial crop and turf uses of IBA result in very low exposures to the environment -- the maximum application rate is 7 mg/acre/crop season (1.7×10^{-5} pounds/acre). By comparison, this rate is five orders of magnitude lower than use rates typical of conventional pesticides and lower than most other biochemicals. Low application rates of IBA to crop land result in correspondingly low environmental concentrations and exposure to nontarget plants and animals. The Agency recognizes that IBA's low application rate alone is not sufficient evidence on which to base an environmental assessment. However, IBA's low application rate combined with its similarity in structure and physiological function to natural plant growth regulators are important factors to consider when assessing potential risks to nontarget terrestrial and aquatic plants and animals.

Indole-3-butyric acid (IBA) is similar in structure and biological activity to the naturally occurring plant growth hormone indole-3-acetic acid (IAA), a principal hormone of higher plants. Compounds similar to IBA (indoleacetamide, indoleacetaldehyde, indoleacetonitrile, and indolepyruvic acid) are intermediate metabolites in the synthesis of IAA from the amino acid tryptophan, which occurs widely in plants, fungi, bacteria, humans and other species. In fact, the average human excretes approximately 7 mg of IAA in urine daily. Given this occurrence of tryptophan and IAA in such a wide diversity of organisms, it is reasonable to assume IAA and similar compounds have metabolic pathways in avian and aquatic species.

Even though specific data have not been submitted, the Agency believes that IBA applied to the environment may be metabolized to IAA and other metabolites by soil, plant, and aquatic microorganisms. The chemical structure of IBA is very similar to IAA, the difference being that the aliphatic side chain contains two additional carbon

atoms. It is reasonable to conclude that a major mechanism of metabolism in the environment is ß-oxidation, a metabolic process in which fatty acids are metabolized by subtraction of two-carbon fragments. This is a common means of microbial metabolism of compounds such as IBA which consist of side chains linked to rings[4]. ß-oxidation of fatty acids also occurs in animal cells[5]. Further, IBA's metabolites and degradates after an application of 7 mg/acre are a negligible incremental contribution to the environment compared to naturally occurring amounts of IAA and related compounds.

For these reasons -- very low application rate, non-lethal mode of action, similar chemical structure and metabolic pathways to other well-known compounds, and natural occurrence in the environment -- the Agency believes that the registered uses of IBA are not likely to pose any significant incremental toxicity to nontarget plants or animals. It is important to note, however, that no data regarding environmental effects have been presented. Therefore, to confirm the presumption of no unreasonable risk to nontarget organisms, the Agency is requiring four basic ecotoxicity studies: acute oral and dietary avian toxicity and freshwater fish and aquatic invertebrate toxicity.

2. Environmental Risk Assessment

Above, the Agency has presented a set of facts and reasonable assumptions about IBA's behavior in organisms and the environment. In summary, 1) IBA is applied to the environment (crop land) in very low amounts resulting in correspondingly low exposures to plants and animals; 2) its mode of biological activity is a plant growth hormone rather than a toxicant or repellant; 3) it is structurally and functionally similar to other naturally occurring compounds which are ubiquitous in biological organisms; and 4) there is some evidence that IBA occurs naturally in plants.

Given these considerations the Agency believes the current uses of IBA will not result in unreasonable risks to the environment. While the Agency does not have toxicity values of IBA for representative avian and aquatic species and certain assumptions have been used to derive its environmental risk conclusion, the Agency nevertheless expects no unreasonable risk from the uses covered by this document. To support this conclusion and confirm related assumptions, the Agency is requiring data from four ecotoxicity studies. Refer to Appendix F for these specific data requirements.

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[4] Alexander, M. 1977. Introduction to soil microbiology. Second edition. John Wiley and Sons, New York.

[5] Lehninger, A.L. 1975. Biochemistry. Second edition. Worth Publishers, Inc. New York.

IV. RISK MANAGEMENT AND REREGISTRATION DECISION FOR INDOLE-3-BUTYRIC ACID

A. Determination of Eligibility

Section 4(g)(2)(A) of FIFRA requires the Agency to determine, after submission of relevant data concerning an active ingredient, whether products containing the active ingredient are eligible for reregistration. The Agency has waived the submission of most generic (i.e., active ingredient specific) data, except for technical chemistry data and acute ecotoxicity data for confirmatory reasons. The Agency has completed its review of the technical chemistry data and other factors and considerations, and has determined that this information is sufficient to support reregistration of all products containing IBA for all uses. Appendix B identifies the generic studies that the Agency reviewed for the determination of reregistration eligibility for IBA.

The Agency therefore finds that products containing only IBA as an active ingredient are eligible for reregistration once the product specific data, confirmatory ecotoxicity data, and amended labeling are received and accepted by the Agency. Products that contain additional active ingredients will be reregistered once the Agency completes eligibility decisions on the other active ingredients and once product specific and amended labeling are received and accepted. The reregistration of particular products is addressed in Section V of this document ("Product Reregistration").

Although the Agency has found that all products containing IBA are eligible for reregistration, it should be understood that the Agency may take appropriate regulatory action and/or require the submission of additional data to support reregistration of products containing IBA, if new information comes to the Agency's attention or if the data requirements for registration change.

V. ACTIONS REQUIRED BY REGISTRANTS

A. Determination of Eligibility

Based on consideration of data and information submitted for the active ingredient, IBA and the registered use patterns, the products containing this active ingredient are eligible for reregistration. Section 4(g)(2)(B) of FIFRA requires that the Agency obtain any needed product-specific data regarding the pesticide following a determination of eligibility. The Agency will review these data and the confirmatory ecotoxicity data and determine whether to reregister individual products.

B. Confirmatory Generic Data Requirements

These data requirements are presented in Appendix F.

C. Product Specific Data Requirements

The product-specific data requirements are stated in Appendix G.

D. Labeling Requirements for End-Use Products

1. The labels and labeling of all products must comply with EPA's current regulations and requirements. Follow the instructions in PR Notice 91-2 (Appendix D) and the Product Reregistration Handbook (Appendix E) with respect to labels and labeling.

2. The labels of IBA products in *Toxicity Category II for primary eye* irritation must bear the signal word "Warning" and must include the following eye protection/protective eyewear statement:

> "Causes substantial but temporary eye injury. Do not get in eyes or on clothing. Wear goggles, face shield or safety glasses. Wash thoroughly with soap and water after handling. Remove contaminated clothing and wash clothing before reuse."
>
> The accompanying statement of practical treatment should read:
>
> "If in eyes: hold eyelids open and flush with a steady, gentle stream of water for 15 minutes. If swallowed: drink promptly a large quantity of milk, egg white, gelatin solution, or if these are not available, large quantities of water. Avoid Alcohol."

APPENDIX A

Indole-3-butyric Acid Use Patterns Subject to Reregistration

APPENDIX A: Case 2330 [Indole-3-butyric acid] Chemical 046701 [Indole-3-butyric acid]

SITE Application Type, Application Timing, Application Equipment	Form	Minimum Application Rate	Maximum Application Rate	Max # Apps	Max. # Apps @ Max. Rate (Days)	Minimum Interval Between Apps. @ Max (Days)	Restricted Interval (Days)	Geographic Limitations	Use Limitation (code)
NON-FOOD/NON-FEED USES - ELIGIBLE FOR REREGISTRATION									
ORNAMENTAL HERBACEOUS PLANTS Use Group(s): Terrestrial Non-Food Crop, Greenhouse Non-Food Crop, Indoor Residential and Outdoor Residential									
Chemigation, Foliar, Irrigation	SC/L	na	Dose cannot be calculated	Not spec	Not spec	Not spec	None	None	
Container plant, Cutting, Not on label	SC/L	na	Dose cannot be calculated	Not spec	Not spec	Not spec	None	None	
Dip, Cutting, Not on label	SC/L	na	Not specified	Not spec	Not spec	Not spec	None	None	
Dip, Cutting, Not on label	WP/D	na	Not specified	Not spec	Not spec	Not spec	None	None	
Dip, Root stock, Not on label	SC/L	na	Not specified	Not spec	Not spec	Not spec	None	None	
Dust, Bulbs, Dust bag	D	na	Not specified	Not spec	Not spec	Not spec	None	None	
Dust, Corms, Dust bag	D	na	Not specified	Not spec	Not spec	Not spec	None	None	
Dust, Cutting, Not on label	D	na	0.001 lb/35,000 cuttings	Not spec	Not spec	Not spec	None	None	

APPENDIX A:Case 2330 [Indole-3-butyric acid] Chemical 046701 [Indole-3-butyric acid]									
SITE Application Type, Application Timing, Application Equipment	Form	Minimum Application Rate	Maximum Application Rate	Max # Apps	Max. # Apps @ Max. Rate (Days)	Minimum Interval Between Apps. @ Max (Days)	Restricted Interval (Days)	Geographic Limitations	Use Limitation (code)
Dust, Root stock, Dust bag	D	na	Not specified	Not spec	Not spec	Not spec	None	None	
Dust, Seed, Dust bag	D	na	Not specified	Not spec	Not spec	Not spec	None	None	
Soil drench, Post planting, Not on label	SC/L	na	Dose cannot be calculated	Not spec	Not spec	7	None	None	
ORNAMENTAL HERBACEOUS PLANTSUse Group(s):Terrestrial Non-Food Crop, Greenhouse Non-Food Crop, Indoor Residential and Outdoor Residential									
Soil drench, Transplant, Not on label	SC/L	na	Dose cannot be calculated	Not spec	Not spec	7	None	None	
Soil treatment, At planting, Not on label	SC/L	na	Dose cannot be calculated	Not spec	Not spec	Not spec	None	None	
Soil treatment, Postplant, Not on label	SC/L	na	Dose cannot be calculated	Not spec	Not spec	Not spec	None	None	
Soil treatment, Pretransplant, Not on label	D	na	Dose cannot be calculated	Not spec	Not spec	Not spec	None	None	
Soil treatment, Pretransplant, Not on label	SC/L	na	Dose cannot be calculated	Not spec	Not spec	Not spec	None	None	
Soil treatment, Not on label, Not on label	SC/L	na	Dose cannot be calculated	Not spec	Not spec	Not spec	None	None	
Spot soil treatment, Plant bed, Not on label	D	na	Dose cannot be calculated	Not spec	Not spec	Not spec	None	None	
Spot soil treatment, Plant bed, Not on label	SC/L	na	Dose cannot be calculated	Not spec	Not spec	Not spec	None	None	
Spot soil treatment, Post planting, Not on label	SC/L	na	Dose cannot be calculated	Not spec	Not spec	Not spec	None	None	

APPENDIX A:Case 2330 [Indole-3-butyric acid] Chemical 046701 [Indole-3-butyric acid]									
SITE Application Type, Application Timing, Application Equipment	Form	Minimum Application Rate	Maximum Application Rate	Max # Apps	Max. # Apps @ Max. Rate (Days)	Minimum Interval Between Appl. @ Max (Days)	Restricted Interval (Days)	Geographic Limitations	Use Limitation (code)
Spot soil treatment, Post planting, Not on label	D	na	Dose cannot be calculated	Not spec	Not spec	Not spec	None	None	

APPENDIX A:Case 2330 [Indole-3-butyric acid] Chemical 046701 [Indole-3-butyric acid]									
SITE Application Type, Application Timing, Application Equipment	Form	Minimum Application Rate	Maximum Application Rate	Max # Apps	Max. # Apps @ Max. Rate (Days)	Minimum Interval Between Apps. @ Max. (Days)	Restricted Interval (Days)	Geographic Limitations	Use Limitation (code)
ORNAMENTAL WOODY SHRUBS AND VINES Use Group(s): Terrestrial Non-Food Crop, Greenhouse Non-Food Crop and Outdoor Residential									
Chemigation, Foliar, Irrigation	SC/L	na	Dose cannot be calculated	Not spec	Not spec	Not spec	None	None	
Dip, Cutting, Not on label	SC/L	na	Dose cannot be calculated	Not spec	Not spec	Not spec	None	None	
Dip, Cutting, Not on label	WP/D	na	Not specified	Not spec	Not spec	Not spec	None	None	
Dust, Cutting, Hand held duster	D	na	Not specified	Not spec	Not spec	Not spec	None	None	
Dust, Cutting, Not on label	D	na	0.001 si lb/35,000 cuttings	Not spec	Not spec	Not spec	None	None	
Soil treatment, At planting, Not on label	SC/L	na	Dose cannot be calculated	Not spec	Not spec	Not spec	None	None	
Soil treatment, Post planting, Not on label	SC/L	na	Dose cannot be calculated	Not spec	Not spec	Not spec	None	None	
Soil treatment, Pretransplant, Not on label	SC/L	na	Dose cannot be calculated	Not spec	Not spec	Not spec	None	None	
Spot soil treatment, Post plant, Not on label	SC/L	na	Dose cannot be calculated	Not spec	Not spec	Not spec	None	None	
Spot soil treatment, Preplant, Not on label	SC/L	na	Dose cannot be calculated	Not spec	Not spec	Not spec	None	None	
Spray, Foliar, Aircraft	SC/L	na	Dose cannot be calculated	Not spec	Not spec	Not spec	None	None	

APPENDIX A: Case 2330 [Indole-3-butyric acid] Chemical 046701 [Indole-3-butyric acid]

SITE Application Type, Application Timing, Application Equipment	Form	Minimum Application Rate	Maximum Application Rate	Max # Apps	Max. # Apps @ Max. Rate (Days)	Minimum Interval Between Apps. @ Max (Days)	Restricted Interval (Days)	Geographic Limitations	Use Limitation (code)
ORNAMENTAL WOODY SHRUBS AND VINES Use Group(s): Terrestrial Non-Food Crop, Greenhouse Non-Food Crop and Outdoor Residential									
Spray, Foliar, Ground	SC/L	na	Dose cannot be calculated	Not spec	Not spec	Not spec	None	None	
ORNAMENTAL AND/OR SHADE TREESUse Group(s):Terrestrial Non-Food Crop, Greenhouse Non-Food Crop and Outdoor Residential									
Dip, Cutting, Not on label	SC/L	na	Dose cannot be calculated	Not spec	Not spec	Not spec	None	None	
Dip, Cutting, Not on label	WP/D	na	Dose cannot be calculated	Not spec	Not spec	Not spec	None	None	
Dust, Cutting, Not on label	D	na	0.0013 lb ai/ 1000 cuttings or not specified	Not spec	Not spec	Not spec	None	None	
Soil treatment, At planting, Not on label	D	na	Dose cannot be calculated	Not spec	Not spec	Not spec	None	None	
Soil treatment, Pretransplant, Not on label	SC/L	na	Dose cannot be calculated	Not spec	Not spec	Not spec	None	None	
Soil treatment, Post planting, Not on label	SC/L	na	Dose cannot be calculated	Not spec	Not spec	Not spec	None	None	
Spot soil treatment, Post plant, Not on label	SC/L	na	Dose cannot be calculated	Not spec	Not spec	Not spec	None	None	
Spot soil treatment, Preplant, Not on label	SC/L	na	Dose cannot be calculated	Not spec	Not spec	Not spec	None	None	
Spray, Foliar, Aircraft	SC/L	na	Dose cannot be calculated	Not spec	Not spec	Not spec	None	None	

APPENDIX A:Case 2330 [Indole-3-butyric acid] Chemical 046701 [Indole-3-butyric acid]									
SITE Application Type, Application Timing, Application Equipment	Form	Minimum Application Rate	Maximum Application Rate	Max # Apps	Max. # Apps @ Max. Rate (Days)	Minimum Interval Between Apps. @ Max (Days)	Restricted Interval (Days)	Geographic Limitations	Use Limitation (code)
ORNAMENTAL AND/OR SHADE TREESUse Group(s):Terrestrial Non-Food Crop, Greenhouse Non-Food Crop and Outdoor Residential									
Spray, Foliar, Ground	SC/L	na	Dose cannot be calculated	Not spec	Not spec	Not spec	None	None	
FOOD/FEED USES - ELIGIBLE FOR REREGISTRATION									
BARLEY (CEREAL GRAIN) Use Group(s): Terrestrial Food Crop and Terrestrial Feed Crop									
Chemigation, Foliar, Irrigation	SC/L	na	not specified	Not spec	Not spec	Not spec	None	None	
Broadcast, Foliar, Aircraft	SC/L	na	0.0000053 lb ai/A	Not spec	Not spec	Not spec	Not Spec	None	
Broadcast, Foliar, Ground	SC/L	na	0.0000053 lb ai/A	Not spec	Not spec	Not spec	Not Spec	None	

APPENDIX A:Case 2330 [Indole-3-butyric acid] Chemical 046701 [Indole-3-butyric acid]									
SITE Application Type, Application Timing, Application Equipment	Form	Minimum Application Rate	Maximum Application Rate	Max # Apps	Max. # Apps @ Max. Rate (Days)	Minimum Interval Between Apps. @ Max (Days)	Restricted Interval (Days)	Geographic Limitations	Use Limitation (code)
BEANS Use Group(s): Terrestrial Food Crop and Terrestrial Feed Crop									
Chemigation, Foliar, Irrigation	SC/L	na	not specified	Not spec	Not spec	Not spec	None	None	
Broadcast, Bloom, Aircraft	SC/L	na	0.0000059 lb ai/A	Not spec	Not spec	Not spec	None	None	
Broadcast, Bloom, Ground	SC/L	na	0.0000059 lb ai/A	Not spec	Not spec	Not spec	None	None	
Broadcast, Foliar, Aircraft	SC/L	na	0.000002 lb ai/A	Not spec	Not spec	7	Not Spec	None	
Broadcast, Foliar, Aircraft	SC/L	na	0.0000026 lb ai/A	Not spec	Not spec	Not spec	Not Spec	None	
Broadcast, Foliar, Ground	SC/L	na	0.000002 lb ai/A	Not spec	Not spec	7	Not Spec	None	
	SC/L	na	0.0000026 lb ai/A	Not spec	Not spec	Not spec	Not Spec	None	

APPENDIX A:Case 2330 [Indole-3-butyric acid] Chemical 046701 [Indole-3-butyric acid]									
SITE Application Type, Application Timing, Application Equipment	Form	Minimum Application Rate	Maximum Application Rate	Max # Apps	Max. # Apps @ Max. Rate (Days)	Minimum Interval Between Apps. @ Max (Days)	Restricted Interval (Days)	Geographic Limitations	Use Limitation (code)
BROCCOLI Use Group(s): Terrestrial Food Crop									
Chemigation, Foliar, Irrigation	SC/L	na	not specified	Not spec	Not spec	Not spec	None	None	
Broadcast, Foliar, Aircraft	SC/L	na	0.0000059 lb ai/A	Not spec	Not spec	14	None	None	
Broadcast, Post transplant, Aircraft	SC/L	na	0.0000059 lb ai/A	Not spec	Not spec	14	None	None	
Broadcast, Foliar, Ground	SC/L	na	0.0000059 lb ai/A	Not spec	Not spec	14	None	None	
Broadcast, Post transplant, Ground	SC/L	na	0.0000059 lb ai/A	Not spec	Not spec	14	None	None	

APPENDIX A:Case 2330 [Indole-3-butyric acid] Chemical 046701 [Indole-3-butyric acid]									
site Application Type, Application Timing, Application Equipment	Form	Minimum Application Rate	Maximum Application Rate	Max # Apps	Max. # Apps @ Max. Rate (Days)	Minimum Interval Between Apps. @ Max (Days)	Restricted Interval (Days)	Geographic Limitations	Use Limitation (calc)
BRUSSELS SPROUTS Use Group(s): Terrestrial Food Crop									
Chemigation, Foliar, Irrigation	SC/L	na	not specified	Not spec	Not spec	Not spec	None	None	
Broadcast, Foliar, Aircraft	SC/L	na	0.0000059 lb ai/A	Not spec	Not spec	14	None	None	
Broadcast, Post transplant, Aircraft	SC/L	na	0.0000059 lb ai/A	Not spec	Not spec	14	None	None	
Broadcast, Foliar, Ground	SC/L	na	0.0000059 lb ai/A	Not spec	Not spec	14	None	None	
Broadcast, Post transplant, Ground	SC/L	na	0.0000059 lb ai/A	Not spec	Not spec	14	None	None	

APPENDIX A:Case 2330 [Indole-3-butyric acid] Chemical 046701 [Indole-3-butyric acid]									
SITE Application Type, Application Timing, Application Equipment	Form	Minimum Application Rate	Maximum Application Rate	Max # Apps	Max. # Apps @ Max. Rate (Days)	Minimum Interval Between Apps. @ Max (Days)	Restricted Interval (Days)	Geographic Limitations	Use Limitation (code)
CABBAGE Use Group(s): Terrestrial Food Crop									
Chemigation, Foliar, Irrigation	SC/L	na	not specified	Not spec	Not spec	Not spec	None	None	
Broadcast, Foliar, Aircraft	SC/L	na	0.0000059 lb ai/A	Not spec	Not spec	14	None	None	
Broadcast, Post transplant, Aircraft	SC/L	na	0.0000059 lb ai/A	Not spec	Not spec	14	None	None	
Broadcast, Foliar, Ground	SC/L	na	0.0000059 lb ai/A	Not spec	Not spec	14	None	None	
Broadcast, Post transplant, Ground	SC/L	na	0.0000059 lb ai/A	Not spec	Not spec	14	None	None	

APPENDIX A:Case 2330 [Indole-3-butyric acid] Chemical 046701 [Indole-3-butyric acid]									
SITE Application Type, Application Timing, Application Equipment	Form	Minimum Application Rate	Maximum Application Rate	Max # Appn	Max. # Appn @ Max. Rate (Days)	Minimum Interval Between Appn. @ Max (Days)	Restricted Interval (Days)	Geographic Limitations	Use Limitation (code)
CAULIFLOWER Use Group(s): Terrestrial Food Crop									
Chemigation, Foliar, Irrigation	SC/L	na	not specified	Not spec	Not spec	Not spec	None	None	
Broadcast, Foliar, Aircraft	SC/L	na	0.0000059 lb ai/A	Not spec	Not spec	14	None	None	
Broadcast, Post transplant, Aircraft	SC/L	na	0.0000059 lb ai/A	Not spec	Not spec	14	None	None	
Broadcast, Foliar, Ground	SC/L	na	0.0000059 lb ai/A	Not spec	Not spec	14	None	None	
Broadcast, Post transplant, Ground	SC/L	na	0.0000059 lb ai/A	Not spec	Not spec	14	None	None	

APPENDIX A: Case 2330 [Indole-3-butyric acid] Chemical 046701 [Indole-3-butyric acid]

SITE Application Type, Application Timing, Application Equipment	Form	Minimum Application Rate	Maximum Application Rate	Max # Apps	Max. # Apps @ Max. Rate (Days)	Minimum Interval Between Apps. @ Max (Days)	Restricted Interval (Days)	Geographic Limitations	Use Limitation (code)
CITRUS FRUITS Use Group(s): Terrestrial Food Crop and Terrestrial Feed Crop									
Chemigation, Foliar, Irrigation	SC/L	na	not specified	Not spec	Not spec	Not spec	None	None	
Dip, Transplants, Not on label	SC/L	na	not specified	Not spec	Not spec	Not spec	None	None	
Directed spray, Transplants, Not on label	SC/L	na	not specified	Not spec	Not spec	Not spec	None	None	
Spray, Foliar, Not on label	SC/L	na	not specified	3/year	Not spec	Not spec	None	None	
CORN, FIELD,SWEET (CEREAL GRAIN) Use Group(s): Terrestrial Food Crop and Terrestrial Feed Crop									
Chemigation, Foliar, Irrigation	SC/L	na	not specified	Not spec	Not spec	Not spec	None	None	
Broadcast, Premergence, Ground	SC/L	na	0.0000078 lb ai/A	1	1	na	None	None	
Broadcast, Premergence, Aircraft	SC/L	na	0.0000078 lb ai/A	1	1	Not spec	None	None	
Band, Foliar, Ground	SC/L	na	0.0000026 lb ai/A	1	1	Not spec	None	None	

APPENDIX A:Case 2330 [Indole-3-butyric acid] Chemical 046701 [Indole-3-butyric acid]									
SITE Application Type, Application Timing, Application Equipment	Form	Minimum Application Rate	Maximum Application Rate	Max # Apps	Max. # Apps @ Max. Rate (Days)	Minimum Interval Between Apps. @ Max (Days)	Restricted Interval (Days)	Geographic Limitations	Use Limitation (code)
COTTON (UNSPECIFIED) Use Group(s): Terrestrial Food Crop and Terrestrial Feed Crop									
Chemigation, Foliar, Irrigation	SC/L	na	not specified	Not spec	Not spec	Not spec	None	None	
Broadcast, In seed furrow, Ground	SC/L	na	0.0000039 lb ai/A	Not spec	Not spec	7	None	None	
Band, At cotyledon, Ground	SC/L	na	0.0000039 lb ai/A	Not spec	Not spec	7	None	None	
Broadcast, Bloom, Aircraft	SC/L	na	0.0000039 lb ai/A	Not spec	Not spec	7	None	None	
Broadcast, Foliar, Aircraft	SC/L	na	0.0000039 lb ai/A	Not spec	Not spec	7	None	None	
Broadcast, Bloom, Ground	SC/L	na	0.0000039 lb ai/A	Not spec	Not spec	7	None	None	
Broadcast, Foliar, Ground	SC/L	na	0.0000039 lb ai/A	Not spec	Not spec	7	None	None	

APPENDIX A:Case 2330 [Indole-3-butyric acid] Chemical 046701 [Indole-3-butyric acid]									
SITE Application Type, Application Timing, Application Equipment	Form	Minimum Application Rate	Maximum Application Rate	Max # Apps.	Max. # Apps @ Max. Rate (Days)	Minimum Interval Between Apps. @ Max (Days)	Restricted Interval (Days)	Geographic Limitations	Use Limitation (units)
CUCUMBER Use Group(s): Terrestrial Food Crop									
Chemigation, Foliar, Irrigation	SC/L	na	not specified	Not spec	Not spec	Not spec	None	None	
Broadcast, Bloom, Aircraft	SC/L	na	0.0000029 lb ai/A	Not spec	Not spec	7	None	None	
Broadcast, Foliar, Aircraft	SC/L	na	0.0000024 lb ai/A	Not spec	Not spec	7	None	None	
Broadcast, Bloom, Ground	SC/L	na	0.0000024 lb ai/A	Not spec	Not spec	7	None	None	
Broadcast, Foliar, Ground	SC/L	na	0.0000024 lb ai/A	Not spec	Not spec	7	None	None	

APPENDIX A:Case 2330 [Indole-3-butyric acid] Chemical 046701 [Indole-3-butyric acid]									
SITE Application Type, Application Timing, Application Equipment	Form	Minimum Application Rate	Maximum Application Rate	Max # Apps	Max. # Apps @ Max. Rate (Days)	Minimum Interval Between Apps. @ Max (Days)	Restricted Interval (Days)	Geographic Limitations	Use Limitation (code)
LETTUCE Use Group(s): Terrestrial Food Crop									
Chemigation, Foliar, Irrigation	SC/L	na	not specified	Not spec	Not spec	Not spec	None	None	
Broadcast, In furrow, Ground	SC/L	na	0.0000059 lb ai/A	Not spec	Not spec	Not spec	None	None	
Broadcast, In furrow, Aircraft	SC/L	na	0.0000059 lb ai/A	Not spec	Not spec	Not spec	None	None	
Band, Foliar, Ground	SC/L	na	0.0000059 lb ai/A	Not spec	Not spec	Not spec	None	None	
Broadcast, Foliar, Aircraft	SC/L	na	0.0000059 lb ai/A	Not spec	Not spec	Not spec	None	None	
Broadcast, Foliar, Ground	SC/L	na	0.0000059 lb ai/A	Not spec	Not spec	Not spec	None	None	

APPENDIX A:Case 2330 [Indole-3-butyric acid] Chemical 046701 [Indole-3-butyric acid]									
SITE Application Type, Application Timing, Application Equipment	Form	Minimum Application Rate	Maximum Application Rate	Max # Apps	Max. # Apps @ Max. Rate (Days)	Minimum Interval Between Apps. @ Max. (Days)	Restricted Interval (Days)	Geographic Limitations	Use Limitation (code)
MELONS Use Group(s): Terrestrial Food Crop									
Chemigation, Foliar, Irrigation	SC/L	na	not specified	Not spec	Not spec	Not spec	None	None	
Broadcast, Foliar, Aircraft	SC/L	na	0.0000029 lb ai/A	2	2	14	None	None	
Broadcast, Foliar, Ground	SC/L	na	0.0000029 lb ai/A	2	2	14	None	None	
MUSTARD CABBAGE (PAKSHOI) Use Group(s): Terrestrial Food Crop									
Band, Foliar, Ground	SC/L	na	0.0000059 lb ai/A	Not spec	Not spec	Not spec	None	None	
Broadcast, Foliar, Aircraft	SC/L	na	0.0000059 lb ai/A	Not spec	Not spec	Not spec	None	None	
Broadcast, Foliar, Ground	SC/L	na	0.0000059 lb ai/A	Not spec	Not spec	Not spec	None	None	
Broadcast, Cutting, Ground	SC/L	na	0.0000059 lb ai/A	Not spec	Not spec	Not spec	None	None	
Broadcast, Cutting, Aircraft	SC/L	na	0.0000059 lb ai/A	Not spec	Not spec	Not spec	None	None	

APPENDIX A:Case 2330 [Indole-3-butyric acid] Chemical 046701 [Indole-3-butyric acid]									
SITE Application Type, Application Timing, Application Equipment	Form	Minimum Application Rate	Maximum Application Rate	Max # Apps	Max. # Apps @ Max. Rate (Days)	Minimum Interval Between Apps. @ Max (Days)	Restricted Interval (Days)	Geographic Limitations	Use Limitation (code)
MUSTARD (LEAFY/STEM CROP) Use Group(s): Terrestrial Food Crop and Terrestrial Feed Crop									
Chemigation, Foliar, Irrigation	SC/L	na	not specified	Not spec	Not spec	Not spec	None	None	
Broadcast, In furrow, Ground	SC/L	na	0.0000059 lb ai/A	Not spec	Not spec	Not spec	None	None	
Band, Foliar, Ground	SC/L	na	0.0000059 lb ai/A	Not spec	Not spec	Not spec	None	None	
Broadcast, Cutting, Aircraft	SC/L	na	0.0000059 lb ai/A	Not spec	Not spec	Not spec	None	None	
Broadcast, Cutting, Ground	SC/L	na	0.0000059 lb ai/A	Not spec	Not spec	Not spec	None	None	

APPENDIX A:Case 2330 [Indole-3-butyric acid] Chemical 046701 [Indole-3-butyric acid]

SITE Application Type, Application Timing, Application Equipment	Form	Minimum Application Rate	Maximum Application Rate	Max # Apps	Max. # Apps @ Max. Rate (Days)	Minimum Interval Between Apps. @ Max (Days)	Restricted Interval (Days)	Geographic Limitations	Use Limitation (acts)
OATS (CEREAL GRAIN) Use Group(s): Terrestrial Food Crop and Terrestrial Feed Crop									
Chemigation, Foliar, Irrigation	SC/L	na	not specified	Not spec	Not spec	Not spec	None	None	
Broadcast, Foliar, Aircraft	SC/L	na	0.0000053 lb ai/A	Not spec	Not spec	Not spec	Not Spec	None	
Broadcast, Foliar, Ground	SC/L	na	0.0000053 lb ai/A	Not spec	Not spec	Not spec	Not Spec	None	
ONION Use Group(s): Terrestrial Food Crop									
Chemigation, Foliar, Irrigation	SC/L	na	not specified	Not spec	Not spec	Not spec	None	None	
Broadcast, Foliar, Aircraft	SC/L	na	not specified	Not spec	Not spec	Not spec	Not Spec	None	
Broadcast, Foliar, Ground	SC/L	na	not specified	Not spec	Not spec	Not spec	Not Spec	None	

APPENDIX A:Case 2330 [Indole-3-butyric acid] Chemical 046701 [Indole-3-butyric acid]									
SITE Application Type, Application Timing, Application Equipment	Form	Minimum Application Rate	Maximum Application Rate	Max # Apps	Max. # Apps @ Max. Rate (Days)	Minimum Interval Between Apps. @ Max (Days)	Restricted Interval (Days)	Geographic Limitations	Use Limitation (code)
ONION (GREEN) (MISCELLANEOUS VEGETABLE) Use Group(s): Terrestrial Food Crop									
Chemigation, Foliar, Irrigation	SC/L	na	not specified	Not spec	Not spec	Not spec	None	None	
Broadcast, Foliar, Aircraft	SC/L	na	not specified	Not spec	Not spec	Not spec	Not Spec	None	
Broadcast, Foliar, Ground	SC/L	na	not specified	Not spec	Not spec	Not spec	Not Spec	None	
PEANUTS Use Group(s): Terrestrial Food Crop and Terrestrial Feed Crop									
Chemigation, Foliar, Irrigation	SC/L	na	not specified	Not spec	Not spec	Not spec	None	None	
Broadcast, At pegging, Aircraft	SC/L	na	0.0000059 lb ai/A	Not spec	Not spec	Not spec	None	None	
Broadcast, At pegging, Ground	SC/L	na	0.0000059 lb ai/A	Not spec	Not spec	Not spec	None	None	

APPENDIX A:Case 2330 [Indole-3-butyric acid] Chemical 046701 [Indole-3-butyric acid]

SITE Application Type, Application Timing, Application Equipment	Form	Minimum Application Rate	Maximum Application Rate	Max # Apps	Max. # Apps @ Max. Rate (Days)	Minimum Interval Between Apps. @ Max (Days)	Restricted Interval (Days)	Geographic Limitations	Use Limitation (code)
PEAS Use Group(s): Terrestrial Food Crop and Terrestrial Feed Crop									
Broadcast, Foliar, Aircraft	SC/L	na	0.000002 lb ai/A	Not spec	Not spec	7	Not Spec	None	
Broadcast, Foliar, Ground	SC/L	na	0.000002 lb ai/A	Not spec	Not spec	7	Not Spec	None	
Broadcast, Bloom, Aircraft	SC/L	na	0.0000059 lb ai/A	Not spec	Not spec	Not spec	None	None	
Broadcast, Bloom, Ground	SC/L	na	0.0000059 lb ai/A	Not spec	Not spec	Not spec	None	None	
PEPPER Use Group(s): Terrestrial Food Crop									
Chemigation, Foliar, Irrigation	SC/L	na	not specified	Not spec	Not spec	Not spec	None	None	
Broadcast, Bloom, Aircraft	SC/L	na	0.000002 lb ai/A	Not spec	Not spec	Not spec	None	None	
Broadcast, Foliar, Aircraft	SC/L	na	0.000002 lb ai/A	Not spec	Not spec	7	None	None	

APPENDIX A:Case 2330 [Indole-3-butyric acid] Chemical 046701 [Indole-3-butyric acid]									
SITE Application Type, Application Timing, Application Equipment	Form	Minimum Application Rate	Maximum Application Rate	Max # Apps	Max. # Apps @ Max. Rate (Days)	Minimum Interval Between Apps. @ Max (Days)	Restricted Interval (Days)	Geographic Limitations	Use Limitation (code)
PEPPER Use Group(s): Terrestrial Food Crop									
Broadcast, Post transplant, Aircraft	SC/L	na	0.000002 lb ai/A	Not spec	Not spec	Not spec	None	None	
Broadcast, Bloom, Ground	SC/L	na	0.000002 lb ai/A	Not spec	Not spec	Not spec	None	None	
Broadcast, Foliar, Ground	SC/L	na	0.000002 lb ai/A	Not spec	Not spec	7	None	None	
Broadcast, Post transplant, Ground	SC/L	na	0.000002 lb ai/A	Not spec	Not spec	Not spec	None	None	
POTATO, WHITE/IRISH Use Group(s): Terrestrial Food Crop and Terrestrial Feed Crop									
Chemigation, Foliar, Irrigation	SC/L	na	not specified	Not spec	Not spec	Not spec	None	None	
Broadcast, Bloom, Aircraft	SC/L	na	0.0000059 lb ai/A	Not spec	Not spec	Not spec	None	None	
Broadcast, Bloom, Ground	SC/L	na	0.0000059 lb ai/A	Not spec	Not spec	Not spec	None	None	

APPENDIX A:Case 2330 [Indole-3-butyric acid] Chemical 046701 [Indole-3-butyric acid]									
SITE Application Type, Application Timing, Application Equipment	Form	Minimum Application Rate	Maximum Application Rate	Max # Appl	Max. # Appl @ Max. Rate (Days)	Minimum Interval Between Appl. @ Max (Days)	Restricted Interval (Days)	Geographic Limitations	Use Limitation (units)
RICE Use Group(s): Terrestrial Food Crop, Terrestrial Feed Crop and Aquatic Food Crop									
Chemigation, Foliar, Irrigation	SC/L	na	not specified	Not spec	Not spec	Not spec	None	None	
Broadcast, Foliar, Aircraft	SC/L	na	0.0000078 lb ai/A	Not spec	Not spec	Not spec	None	None	
Broadcast, Foliar, Ground	SC/L	na	0.0000078 lb ai/A	Not spec	Not spec	Not spec	None	None	
RYE (CEREAL GRAIN) Use Group(s): Terrestrial Food Crop and Terrestrial Feed Crop									
Chemigation, Foliar, Irrigation	SC/L	na	not specified	Not spec	Not spec	Not spec	None	None	
Broadcast, Foliar, Aircraft	SC/L	na	0.0000053 lb ai/A	Not spec	Not spec	Not spec	Not Spec	None	
Broadcast, Foliar, Ground	SC/L	na	0.0000053 lb ai/A	Not spec	Not spec	Not spec	Not Spec	None	

APPENDIX A:Case 2330 [Indole-3-butyric acid] Chemical 046701 [Indole-3-butyric acid]									
SITE Application Type, Application Timing, Application Equipment	Form	Minimum Application Rate	Maximum Application Rate	Max # Apps	Max. # Apps @ Max. Rate (Days)	Minimum Interval Between Apps. @ Max (Days)	Restricted Interval (Days)	Geographic Limitations	Use Limitation (code)
SORGHUM Use Group(s): Terrestrial Food Crop and Terrestrial Feed Crop									
Chemigation, Foliar, Irrigation	SC/L	na	not specified	Not spec	Not spec	Not spec	None	None	
Broadcast, In furrow, Ground	SC/L	na	0.0000078 lb ai/A	Not spec	Not spec	Not spec	None	None	
Broadcast, Foliar, Aircraft	SC/L	na	0.0000078 lb ai/A	Not spec	Not spec	Not spec	None	None	
Broadcast, Foliar, Ground	SC/L	na	0.0000078 lb ai/A	Not spec	Not spec	Not spec	None	None	

APPENDIX A:Case 2330 [Indole-3-butyric acid] Chemical 046701 [Indole-3-butyric acid]									
SITE Application Type, Application Timing, Application Equipment	Form	Minimum Application Rate	Maximum Application Rate	Max # Apps	Max. # Apps @ Max. Rate (Days)	Minimum Interval Between Appns. @ Max. (Days)	Restricted Interval (Days)	Geographic Limitations	Use Limitation (code)
SOYBEANS (UNSPECIFIED) Use Group(s): Terrestrial Food Crop and Terrestrial Feed Crop									
Chemigation, Foliar, Irrigation	SC/L	na	not specified	Not spec	Not spec	Not spec	None	None	
Broadcast, Preplant incorporated, Ground	SC/L	na	0.0000059 lb ai/A	Not spec	Not spec	Not spec	None	None	
Broadcast, Bloom, Aircraft	SC/L	na	0.0000059 lb ai/A	Not spec	Not spec	Not spec	None	None	
Broadcast, Bloom, Ground	SC/L	na	0.0000059 lb ai/A	Not spec	Not spec	Not spec	None	None	

APPENDIX A:Case 2330 [Indole-3-butyric acid] Chemical 046701 [Indole-3-butyric acid]

SITE Application Type, Application Timing, Application Equipment	Form	Minimum Application Rate	Maximum Application Rate	Max # Apps	Max. # Apps @ Max. Rate (Days)	Minimum Interval Between Apps. @ Max (Days)	Restricted Interval (Days)	Geographic Limitations	Use Limitation (code)
SPINACH Use Group(s): Terrestrial Food Crop									
Chemigation, Foliar, Irrigation	SC/L	na	not specified	Not spec	Not spec	Not spec	None	None	
Broadcast, In furrow, Ground	SC/L	na	0.0000059 lb ai/A	Not spec	Not spec	Not spec	None	None	
Band, Foliar, Ground	SC/L	na	0.0000059 lb ai/A	Not spec	Not spec	Not spec	None	None	
Broadcast, Cutting, Aircraft	SC/L	na	0.0000059 lb ai/A	Not spec	Not spec	Not spec	None	None	
Broadcast, Cutting, Ground	SC/L	na	0.0000059 lb ai/A	Not spec	Not spec	Not spec	None	None	
SQUASH (SUMMER/WINTER/ ZUCCHINI) Use Group(s): Terrestrial Food Crop									
Chemigation, Foliar, Irrigation	SC/L	na	not specified	Not spec	Not spec	Not spec	None	None	
Broadcast, Bloom, Aircraft	SC/L	na	0.0000029 lb ai/A	Not spec	Not spec	7	None	None	

APPENDIX A:Case 2330 [Indole-3-butyric acid] Chemical 046701 [Indole-3-butyric acid]									
SITE Application Type, Application Timing, Application Equipment	Form	Minimum Application Rate	Maximum Application Rate	Max # Apps	Max. # Apps @ Max. Rate (Days)	Minimum Interval Between Apps. @ Max (Days)	Restricted Interval (Days)	Geographic Limitations	Use Limitation (code)
SQUASH (SUMMER/WINTER/ ZUCCHINI) Use Group(s): Terrestrial Food Crop									
Broadcast, Foliar, Aircraft	SC/L	na	0.0000029 lb ai/A	Not spec	Not spec	7	None	None	
Broadcast, Bloom, Ground	SC/L	na	0.0000029 lb ai/A	Not spec	Not spec	7	None	None	
Broadcast, Foliar, Ground	SC/L	na	0.0000029 lb ai/A	Not spec	Not spec	7	None	None	
STRAWBERRY Use Group(s): Terrestrial Food Crop									
Chemigation, Foliar, Irrigation	SC/L	na	not specified	Not spec	Not spec	Not spec	None	None	
Broadcast, Bloom, Aircraft	SC/L	na	0.0000029 lb ai/A	Not spec	Not spec	Not spec	None	None	
Broadcast, Bloom, Ground	SC/L	na	0.0000029 lb ai/A	Not spec	Not spec	Not spec	None	None	

APPENDIX A: Case 2330 [Indole-3-butyric acid] Chemical 046701 [Indole-3-butyric acid]

SITE Application Type, Application Timing, Application Equipment	Form	Minimum Application Rate	Maximum Application Rate	Max # Apps	Max. # Apps @ Max. Rate (Days)	Minimum Interval Between Apps. @ Max (Days)	Restricted Interval (Days)	Geographic Limitations	Use Limitation (code)
SUGAR BEET (SUGAR CROP) Use Group(s): Terrestrial Food Crop and Terrestrial Feed Crop									
Chemigation, Foliar, Irrigation	SC/L	na	not specified	Not spec	Not spec	Not spec	None	None	
Broadcast, Foliar, Aircraft	SC/L	na	0.000002 lb ai/A	2	Not spec	30	None	None	
Broadcast, Foliar, Ground	SC/L	na	0.000002 lb ai/A	2	Not spec	30	None	None	
SUGARCANE (SUGAR CROP) Use Group(s): Terrestrial Food Crop and Terrestrial Feed Crop									
Chemigation, Foliar, Irrigation	SC/L	na	not specified	Not spec	Not spec	Not spec	None	None	
Soil sidedress treatment, Foliar, Ground	SC/L	na	0.0000078 lb ai/A	Not spec	Not spec	Not spec	None	None	

APPENDIX A:Case 2330 [Indole-3-buty... ...ical 046701 [Indole-3-butyric acid]									
SITE Application Type, Application Timing, Application Equipment	Form	Minimum Application Rate	Maximum Application Rate	Max # Apps	Max. # Apps @ Max. Rate (Days)	Minimum Interval Between Apps. @ Max (Days)	Restricted Interval (Days)	Geographic Limitations	Use Limitation (code)
TOMATO Use Group(s): Terrestrial Food Crop and Terrestrial Feed Crop									
Chemigation, Foliar, Irrigation	SC/L	na	not specified	Not spec	Not spec	Not spec	None	None	
Band, Post transplant, Ground	SC/L	na	0.0000013 lb ai/A	Not spec	Not spec	Not spec	None	None	
Broadcast, Bloom, Aircraft	SC/L	na	0.0000059 lb ai/A	Not spec	Not spec	Not spec	None	None	
Broadcast, Foliar, Aircraft	SC/L	na	0.000002 lb ai/A	Not spec	Not spec	7	None	None	
Broadcast, Post transplant, Aircraft	SC/L	na	0.000002 lb ai/A	Not spec	Not spec	Not spec	None	None	
Broadcast, Bloom, Ground	SC/L	na	0.0000059 lb ai/A	Not spec	Not spec	Not spec	None	None	
Broadcast, Foliar, Ground	SC/L	na	0.000002 lb ai/A	Not spec	Not spec	7	None	None	
Broadcast, Post transplant, Ground	SC/L	na	0.000002 lb ai/A	Not spec	Not spec	Not spec	None	None	

APPENDIX A: Case 2330 [Indole-3-butyric acid] Chemical 046701 [Indole-3-butyric acid]

SITE Application Type, Application Timing, Application Equipment	Form	Minimum Application Rate	Maximum Application Rate	Max # Apps	Max. # Apps @ Max. Rate (Days)	Minimum Interval Between Apps. @ Max (Days)	Restricted Interval (Days)	Geographic Limitations	Use Limitation (mds)
TURNIP Use Group(s): Terrestrial Food Crop and Terrestrial Feed Crop									
Chemigation, Foliar, Irrigation	SC/L	na	not specified	Not spec	Not spec	Not spec	None	None	
Band, Foliar, Ground	SC/L	na	0.0000013 lb ai/A	Not spec	Not spec	Not spec	None	None	
In furrow, At planting, Ground	SC/L	na	0.0000013 lb ai/A	Not spec	Not spec	Not spec	None	None	
Broadcast, Foliar, Aircraft	SC/L	na	0.0000059 lb ai/A	Not spec	Not spec	Not spec	None	None	
Broadcast, Foliar, Ground	SC/L	na	0.0000059 lb ai/A	Not spec	Not spec	Not spec	None	None	

APPENDIX A:Case 2330 [Indole-3-butyric acid] Chemical 046701 [Indole-3-butyric acid]									
SITE Application Type, Application Timing, Application Equipment	Form	Minimum Application Rate	Maximum Application Rate	Max # Apps	Max. # Apps @ Max. Rate (Days)	Minimum Interval Between Apps. @ Max (Days)	Restricted Interval (Days)	Geographic Limitations	Use Limitation (code)
WHEAT (CEREAL GRAIN) Use Group(s): Terrestrial Food Crop and Terrestrial Feed Crop									
Chemigation, Foliar, Irrigation	SC/L	na	not specified	Not spec	Not spec	Not spec	None	None	
Broadcast, Foliar, Aircraft	SC/L	na	0.0000053 lb ai/A	Not spec	Not spec	Not spec	Not Spec	None	
Broadcast, Foliar, Ground	SC/L	na	0.0000053 lb ai/A	Not spec	Not spec	Not spec	Not Spec	None	
NON-FOOD/NON-FEED USES - ELIGIBLE FOR REREGISTRATION									
GOLF COURSE TURF Use Group(s): Terrestrial Non-Food Crop									
Chemigation, Foliar, Irrigation	SC/L	na	not specified	Not spec	Not spec	Not spec	None	None	
Broadcast, Foliar, Ground	SC/L	na	.000017 lb ai/A	Not spec	Not spec	30	none	none	

APPENDIX A:Case 2330 [Indole-3-butyric acid] Chemical 046701 [Indole-3-butyric acid]									
SITE Application Type, Application Timing, Application Equipment	Form	Minimum Application Rate	Maximum Application Rate	Max # Apps	Max. # Apps @ Max. Rate (Days)	Minimum Interval Between Apps. @ Max (Days)	Restricted Interval (Days)	Geographic Limitations	Use Limitation (code)
ORNAMENTAL LAWNS AND TURF Use Group(s): Terrestrial Non-Food Crop and Outdoor Residential									
Chemigation, Foliar, Irrigation	SC/L	na	not specified	Not spec	Not spec	Not spec	None	None	
Broadcast, Established lawns, Ground	SC/L	na	0.0000085 lb ai/A	Not spec	Not spec	Not spec	None	None	
Broadcast, Preharvest, Ground	SC/L	na	0.0000078 lb ai/A	Not spec	Not spec	Not spec	None	None	
Spray, Foliar, Ground	SC/L	na	0.000014 lb ai/A	Not spec	Not spec	30	None	None	
	SC/L	na	0.000017 lb ai/A	2	2	30	None	None	

Abbreviations used

Header: max=maximum; min=minimum; apps=applications; not spec=not specified; na=not applicable
Form: D=dust; SC/L=soluble concentrate/liquid; WP/D=wettable powder dust;
Rate: ai=active ingredient; A=acre; lb=pound

APPENDIX B

Generic Data Requirements for Reregistration of Indole 3-Butyric Acid and Data Citations Supporting Reregistration

GUIDE TO APPENDIX B

Appendix B contains listings of data requirements which support the reregistration for the pesticide covered by this Reregistration Eligibility Document.

Appendix B contains generic data requirements that apply to the pesticide in all products, including data requirements for which a "typical formulation" is the test substance.

The data tables generally are organized according to the following format:

1. Data Requirement (Column 1). The data requirements are listed in the order in which they appear in 40 CFR Part 158. The reference numbers accompanying each test refer to the test protocols set out in the Pesticide Assessment Guidelines, which are available from the National Technical Information Service, 5285 Port Royal Road, Springfield, VA 22161.

3. Use Pattern (Column 2). This colun indicates the use patterns to which the data requirement applies. The following letter designations are used for the given use patterns: C (Terrestrial nonfood); I (Greenhouse nonfood); K (Residential); O (Indoor residential)

2. Bibliographic citation (Column 3). If the EPA has acceptable data in its files, this column lists the identifying number of each study. This normally is the Master Record Identification (MRID) number, but may be a GS number if no MRID number has been assigned. Refer to the Bibliography Appendices for a complete citation of the study.

APPENDIX B

Data Supporting Guideline Requirements for the Reregistration of Indole-3-Butyric Acid

Guideline Citation	Title of study	Use Pattern	Citation
§158.690 Product Chemistry			
151-10	Product Identity	CIKO	41584401
151-11	Manufacturing Process	CIKO	41584401
151-12	Discussion of Formation	CIKO	41584401
151-13	Analysis of samples	CIKO	41584402
151-15	Certification of limits	CIKO	41584402
151-16	Analytical Method	CIKO	41584402
151-17(a)	Color	CIKO	41584403
151-17(b)	Physical State	CIKO	41584403
151-17(c)	Odor	CIKO	41584403

151-17(d)	Melting Point	CIKO	41584403
151-17(e)	Boiling	CIKO	41584403
151-17(f)	Density	CIKO	41584403
151-17(g)	Solubility	CIKO	41584403
151-17(h)	Vapor Pressure	CIKO	41584403
151-17(i)	pH	CIKO	41584403
151-17(j)	Stability	CIKO	41584403
151-17(p)	Octanol/water partition	CIKO	41584403

APPENDIX B

Data Supporting Guideline Requirements for the Reregistration of Indole-3-Butyric Acid

Guideline Citation	Title of Study	Use Pattern	Citation
§158.690 Toxicology			
152-11	Acute Oral	CIKO	Waived
152-11	Acute dermal	CIKO	Waived
152-12	Acute inhalation	CIKO	Waived
152-21	90-day dermal	CIKO	Waived
152-23	Teratogenicity	CIKO	Waived
152-19	Mutagenicity	CIKO	Waived

APPENDIX C

Citations Considered to be Part of the Data Base Supporting the Reregistration of Indole-3-Butyric Acid

GUIDE TO APPENDIX C

1.
CONTENT OF BIBLIOGRAPHY. This bibliography contains citations of all studies considered relevant by EPA in arriving at the positions and conclusions stated elsewhere in the Reregistration Eligibility Document. Primary sources for studies in this bibliography have been the body of data submitted to EPA and its predecessor agencies in support of past regulatory decisions. Selections from other sources including the published literature, in those instances where they have been considered, will be included.

2.
UNITS OF ENTRY. The unit of entry in this bibliography is called a "study". In the case of published materials, this corresponds closely to an article. In the case of unpublished materials submitted to the Agency, the Agency has sought to identify documents at a level parallel to the published article from within the typically larger volumes in which they were submitted. The resulting "studies" generally have a distinct title (or at least a single subject), can stand alone for purposes of review, and can be described with a conventional bibliographic citation. The Agency has attempted also to unite basic documents and commentaries upon them, treating them as a single study.

3.
IDENTIFICATION OF ENTRIES. The entries in this bibliography are sorted numerically by Master Record Identifier number, or "MRID". This number is unique to the citation, and should be used at any time specific reference is required. It is not related to the six-digit "Accession Number" which has been used to identify volumes of submitted studies; see paragraph 4(d)(4) below for further explanation. In a few cases, entries added to the bibliography late in the review may be preceded by a nine-character temporary identifier. These entries are listed after all MRID entries. This temporary identifier number also is to be used whenever specific reference is needed.

4.
FORM OF ENTRY. In addition to the MRID, each entry consists of a citation containing standard elements followed, in the case of material submitted to EPA, by a description of the earliest known submission. Bibliographic conventions used reflect the standards of the American National Standards Institute (ANSI), expanded to provide for certain special needs.

a.
Author. Whenever the Agency could confidently identify one, the Agency has chosen to show a personal author. When no individual was identified, the Agency has shown an identifiable laboratory or testing facility as author. As a last resort, the Agency has shown the first submitter as author.

b.
Document date. When the date appears as four digits with no question marks, the Agency took it directly from the document. When a four-digit date is followed by a question mark, the bibliographer deduced the date from evidence in the document. When the date appears as (19??), the Agency was unable to determine or estimate the date of the document.

c.
Title. In some cases, it has been necessary for Agency bibliographers to create or enhance a document title. Any such editorial insertions are contained between square brackets.

d.
Trailing parentheses. For studies submitted to the Agency in the past, the trailing parentheses include (in addition to any self-explanatory text) the following elements describing the earliest known submission:

(1)
Submission date. The date of the earliest known submission appears immediately following the word "received."

(2)
Administrative number. The next element, immediately following the word "under," is the registration number, experimental use permit number, petition number, or other administrative number associated with the earliest known submission.

(3)
Submitter. The third element is the submitter, following the phrase "submitted by." When authorship is defaulted to the submitter, this element is omitted.

(4)
Volume Identification (Accession Numbers). The final element in the trailing parentheses identifies the EPA accession number of the volume in which the original submission of the study appears. The six-digit accession number follows the symbol "CDL," standing for "Company Data Library." This accession number is in turn followed by an alphabetic suffix which shows the relative position of the study within the volume. For example, within accession number 123456, the first study would be 123456-A; the second, 123456-B; the 26th, 123456-Z; and the 27th, 123456-AA.

APPENDIX C

INDOLE-3-BUTYRIC ACID BIBLIOGRAPHY

MRID Citation

41193801 Syntex Corp. (1989) Indole-3-butyric Acid: Product Identity and Composition. Unpublished study. 56 p.

41584401 Syntex Corp. (1990) Addendum to Guideline 61... per EPA request 1/29/90: Indole-3-Butyric Acid, Product Identity and Composition. 17p.

41193701 Syntex Corp. (1989) Indole-3-Butyric Acid: Analysis and Certification of Product Ingredients. Unpublished study. 20 p.

41584402 *Syntex Corp. (1990) Addendum to Guideline 62... per EPA request of 1/29/90:* Indole-3-Butyric Acid, Analysis and of Product Ingredients: 20 p.

41193702 Syntex Corp. (1989) Indole-3-butyric Acid: Physical and Chemical Characteristics. Unpublished study. 3 p.

41584403 Syntex Corp. (1990) Addendum to Guideline 63 ...per EPA request of 1/29/90: Indole-3-butyric Acid: Physical and Chemical Characteristics. 55 p.

Indole-3-butyric acid (046701) Registrants

Related Information

- Regulating Biopesticides
- Active Ingredient Index

Information related to this page:

- Factsheet
- Federal Register Notices
- Products
- Registrants
- Technical Document (PDF)
  (60 pp, 1.1 MB about PDF)

Company Number	Company Name	Address
009779	AGRILIANCE, LLC	PO BOX 64089 ST, PAUL, MN 55164
066330	ARYSTA LIFESCIENCE NORTH AMERICA CORPORATION	PARK WEST II 15401 WESTON PKWY SUITE 150 CARY, NC 27513
000004	BONIDE PRODUCTS, INC.	6301 SUTLIFF ROAD ORISKANY, NY 13424
008281	BROOKER CHEMICAL CORP	PO BOX 5096 CHATSWORTH, CA 91313
080518	CYTOTEK ENTERPRISES, INC., C/O PYXIS REGULATORY CONSULTING	4110 136TH ST, NW GIG HARBOR, WA 98332
000352	E. I. DU PONT DE NEMOURS AND CO., INC.	STINE-HASKELL RESEARCH CENTER/ 1090 ELKTON RD NEWARK, DE 19714
064014	FLORIDA SILVICS INC	950 S.E. 215TH AVE. MORRISTON, FL 32668
000869	GREEN LIGHT COMPANY	PO BOX 17985 SAN ANTONIO, TX 78217

070908	GROW MORE INC	15600 NEW CENTURY DR GARDENA, CA 90248
079664	GROWTH TECHNOLOGY LTD.	UNIT 66, TAUNTON TRADING ESTATE TAUNTON TA 2 6RX
005905	HELENA CHEMICAL CO	225 SCHILLING BOULEVARD, SUITE 300 COLLIERVILLE, TN 38017
063310	HORTUS USA CORP	245 W 24TH ST NEW YORK, NY 10011
082437	K & W AGRICHEMICALS, INC.	9127 HWAY 431 SOUTH, STE D OWENS CROSS ROADS, AL 35763
034704	LOVELAND PRODUCTS, INC.	PO BOX 1286 GREELEY, CO 80632
072639	LT BIOSYN INC.	11324 17TH AVENUE COURT NW GIG HARBOR, WA 98332
000072	MILLER CHEMICAL AND FERTILIZERMILLER CHEMICAL AND FERTILIZER CORPORATION	PO BOX 333 HANOVER, PA 17331
070127	NOVOZYMES BIOLOGICALS, INC.	111 KESLER MILL RD SALEM, VA 24153
059807	OHP, INC.	PO BOX 230 MAINLAND, PA 19451
059807	OHP, INC.	PO BOX 230 MAINLAND, PA 19451
058199	P.B.T., INC.	HC 66, BOX 74 DEMING, NM 88030
083527	RAPIDGROW INDUSTRIES, INC	12208 QUINQUE LANE CLIFTON, VA 20124

073304	REACTIMEX, S.D. DE C.V.	AVE. INDUSTRIAS 120 PTE. "C" APDO. MONTERREY, N.L.
000572	ROCKLAND CHEMICAL CORP.	71 CAROLYN BLVD. FARMINGDALE, NY 11735
057538	STOLLER ENTERPRISES INC	4001 W. SAM HOUSTON PKWY NORTH, SUITE 100 HOUSTON, TX 77043
075851	TECHNAFLORA PLANT PRODUCTS LTD.	1990 OLD BRIDGE ROAD, SUITE 201 LAKE RIDGE, VA 22192
007401	VOLUNTARY PURCHASING GROUP INC	PO BOX 460 BONHAM, TX 75418

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Indole-3-butyric acid (046701) Products

Related Information

- Regulating Biopesticides
- Active Ingredient Index

Information related to this page:

- Factsheet
- Federal Register Notices
- Products
- Registrants
- Technical Document (PDF)
  (60 pp, 1.1 MB about PDF)

Registration Number	Product Name
000004-00431	BONIDE ROOT & GROW
000072-00577	MILLER 2360
000352-00702	GRIFFIN EARLY HARVEST PGR
000572-00348	HORMO ROOT A
000869-00060	GREEN LIGHT ROOT STIMULATOR AND STARTER SOLUTION
005905-00540	ASSET PGR
007401-00252	FERTI=LOME ROOT STIMULATOR & PLANT STARTER SOLUTION
007401-00343	FERTI-LOME ROOTING POWDER
007401-00465	FERTI-LOME TOMATO & PEPPER SET II
008281-00001	HORMEX ROOTING POWDER NO. 8
008281-00002	HORMEX ROOTING POWDER NO. 16
008281-00003	HORMEX ROOTING POWDER NO. 3
008281-00004	HORMEX ROOTING POWDER NO. 30
008281-00005	HORMEX ROOTING POWDER NO. 45
008281-00006	HORMEX ROOTING POWDER NO. 1
009779-00313	CROP BOOSTER PLUS 12-8-8
009779-00321	MAXON II.
009779-00334	MAXON IV
009779-00335	SUPPER LAGNIAPPE ("SUPER L")

034704-00971	EARLY HARVEST SPEED
034704-00972	EARLY HARVEST EHDST DRY HOPPER BOX SEED DRESSING
057538-00013	STIMULATE YIELD ENHANCER
057538-00014	FOLI-ZYME GA
057538-00017	STIMULATE PLUS YIELD ENHANCER
057538-00019	VIGOR S
057538-00027	STOLLER TECHNICAL INDOLE-3-BUTYRIC ACID
058199-00007	CYTOPLEX HMS
059807-00002	HORMODIN 2
059807-00003	HORMODIN 3
059807-00004	HORMODIN 1
063310-00008	RHIZOPON AA WATER SOLUBLE TABLETS
063310-00019	RHIZOPON AA #1 (0.1)
063310-00020	RHIZOPON AA #2 (0.3)
063310-00021	RHIZOPON AA #3 (0.8)
063310-00022	IBA WATER SOLUBLE SALTS (20%)
064014-00013	SNIPPER
066330-00246	PGR-IV GRANULE
066330-00247	TOMATO & PEPPER FRUIT SET
066330-00257	BOLL-SET
066330-00265	INDOLE-3-BUTYRIC ACID TECHNICAL
066330-00267	PGR-IV CONCENTRATE
066330-00272	PGR-IV PLUS
066330-00275	PGR-IV MUP
066330-00290	PGR-IV ST DUST
070127-00002	NOVOZYMES BIOFUNGICIDE GREEN RELEAF 710-140
070127-00003	NOVOZYMES BIOFUNGICIDE 145F
070908-00002	GROW MORE RAPID ROOT

072639-00001	LT BIOSYN, INC. TECHNICAL 3-INDOLEBUTYRIC ACID
072639-00009	ROOTGRO
072639-00011	GOLDENGRO TM R
072639-00012	MegaGro L
073304-00001	INDOLE-3-BUTYRIC ACID
075851-00002	ROOTECH CLONING GEL
079664-00001	CLONEX ROOTING GEL
080518-00001	ROUSE
080518-00002	AGRA-ROUSE
082437-00002	5-15-5 WITH GRO-ROOT XTRA (GRX)
083527-00001	SUPERNATURAL BRAND ROOTBURST POWDER

Local Navigation

Hortus USA Corp.

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Primary Supplier of Plant Rooting Products

August 29, 2008

Program Manager
USDA/AMS/TM/NOP
Room 4008-So, Ag Stop 0268
1400 Independence Ave., SW
Washington DC 20250
Phone 202-720-3252
Fax 202-720-7808

Dear Program Manager

We sent to you and you received by FEDEX our Petition of substance for inclusion on the National List of Substances Allowed on Organic Production and Handling. A copy of the PETITION is attached. The additional information package which was sent with the original package is not hereby included.

> Subject active ingredient substance: Indole-3-butyric acid, CAS number 133-32-4. Requested as national list substance: As Plant Growth Regulators. Indole-3-butyric acid (IBA).

We are now sending on the following page additional information from US EPA FIFRA which applies to the tolerance for the active ingredient Indole-3-butyric acid

Regards

Joel Kroin
President

[Code of Federal Regulations]
[Title 40, Volume 20]
[Revised as of July 1, 2001]
From the U.S. Government Printing Office via GPO Access
[CITE: 40CFR180.1158]

TITLE 40--PROTECTION OF ENVIRONMENT

CHAPTER I--ENVIRONMENTAL PROTECTION AGENCY--(Continued)

PART 180--TOLERANCES AND EXEMPTIONS FROM TOLERANCES FOR *PESTICIDE CHEMICALS* IN FOOD--Table of Contents

Subpart D--Exemptions From Tolerances

Sec. 180.1158 Auxins; exemption from the requirement of a tolerance.

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.

[64 FR 31505, June 11, 1999]

the expression of the genetic material encoding the CryIA(b) delta-endotoxin, such as promoters, terminators, and enhancers.

[60 FR 42446, Aug. 16, 1995]

§ 180.1153 Lepidopteran pheromones; exemption from the requirement of a tolerance.

Lepidopteran pheromones that are naturally occurring compounds, or identical or substantially similar synthetic compounds, designated by an unbranched aliphatic chain (between 9 and 18 carbons) ending in an alcohol, aldehyde or acetate functional group and containing up to 3 double bonds in the aliphatic backbone, are exempt from the requirement of a tolerance in or on all raw agricultural commodities. This exemption pertains to only those situations when the pheromone is applied to growing crops at a rate not to exceed 150 grams active ingredient/acre/year in accordance with good agricultural practices.

[60 FR 45062, Aug. 30, 1995]

§ 180.1154 CryIA(c) and CryIC derived delta-endotoxins of Bacillus thuringiensis var. kurstaki encapsulated in killed Pseudomonas fluorescens, and the expression plasmid and cloning vector genetic constructs.

CryIA(c) and CryIC derived delta-endotoxins of *Bacillus thuringiensis* var. *kurstaki* encapsulated in killed *Pseudomonas fluorescens* and the expression plasmid and cloning vector genetic constructs are exempt from the requirement of a tolerance when used in or on all raw agricultural commodities.

[60 FR 47489, Sept. 13, 1995]

§ 180.1155 Bacillus thuringiensis subspecies Kurstaki CryIA(c) and the genetic material necessary for its production in all plants; exemption from the requirement of a tolerance.

Bacillus thuringiensis subspecies *kurstaki* CryIA(c) delta-endotoxin and the genetic material necessary for its production in all plants are exempt from the requirement of a tolerance when used as plant-pesticides in all plant raw agricultural commodities. "Genetic material necessary for its production" means the genetic material which comprise genetic material encoding the CryIA(c) delta-endotoxin and its regulatory regions. "Regulatory regions" are the genetic material that control the expression of the genetic material encoding the CryIA(c) delta-endotoxin, such as promoters, terminators, and enhancers.

[62 FR 17722, Apr. 11, 1997]

§ 180.1156 Cinnamaldehyde; exemption from the requirement of a tolerance.

Cinnamaldehyde (3-phenyl-2-propenal) is exempted from the requirement of a tolerance in or on all food commodities, when used as a fungicide, insecticide, and algaecide in accordance with good agricultual practices.

[64 FR 7804, Feb. 17, 1999; 64 FR 14099, Mar. 24, 1999]

§ 180.1157 Cytokinins; exemption from the requirement of a tolerance.

An exemption from the requirement of a tolerance is established for residues of cytokinins (specifically: aqueous extract of seaweed meal and kinetin) 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.

[64 FR 31505, June 11, 1999]

§ 180.1158 Auxins; exemption from the requirement of a tolerance.

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.

[64 FR 31505, June 11, 1999]

§ 180.1159 Pelargonic acid; exemption from the requirement of tolerances.

(a) An exemption from the requirement of a tolerance is established for residues of pelargonic acid in or on all food commodities when used as a plant

Hortus USA Corp.

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Primary Supplier of Plant Rooting Products

August 18, 2008

Program Manager
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Washington DC 20250
Phone 202-720-3252
Fax 202-720-7808

Dear Program Manager

Please find attached our Petition of substance for inclusion on the National List of Substances Allowed on Organic Production and Handling.

> Subject active ingredient substance: Indole-3-butyric acid, CAS number 133-32-4.
> Requested as national list substance: As Plant Growth Regulators. Indole-3-butyric acid (IBA).

If you have any questions please contact me directly.

Regards

Joel Kroin
President

TO:

Officer:	Program Manager
Agency:	USDA/AMS/TM/NOP
Address:	Room 4008-So, Ag Stop 0268 1400 Independence Ave., SW Washington DC 20250
Phone:	202-720-3252
Fax:	202-720-7808

FROM PETITIONER:

Contact person:	Joel Kroin
Title:	President
Company:	Hortus USA Corp.
Address:	245 West 24th Street New York NY 10011
Email address:	support@hortus.com
Phone:	212-929-0927
Fax:	212-624-0202

DATE:

Submission: August 19, 2008

PETITION:

Petition of substances fo inclusion on the National List of Substances allowed on Organic Production and Handling
(7 CFR Part 205, Guidelines on procedures for submitting National List Petitions: AMS-TM-06-0223; TM-06-12)

SUBJECT ACTIVE INGREDIENT SUBSTANCE:

Indole-3-butyric acid CAS number 133-32-4
Empirical formula C12 H13 NO2

REQUESTED AS NATIONAL LIST SUBSTANCE:

As plant growth regulators. Indole-3-butyric acid (IBA)

INTRODUCTION

Reference:
US EPA Indole-3-butyric acid (046701) Fact Sheet issued August 1, 2000 (attached)

Indole-3-butyric acid enhances the growth and development of food crops and ornamentals when applied to soil, cuttings, or leaves. Because it is similar in structure to naturally occurring substances and is used in tiny amounts, this plant growth regulator poses no known risks to humans or the environment.

From the US EPA IBA Fact sheet: Description of the Active Ingredient

Indole-3-butyric acid is a substance that is closely related in structure and function to a natural growth regulator found in plants. Indole-3-butyric acid is used on many crops and ornamentals to promote growth and development of roots, flowers and fruits, and to increase crop yields. Growers find it more effective and efficient than its natural counterpart because plants cannot break it down as quickly. No harm to humans or the environment is expected to result from use of Indole-3-butyric acid.

From the US EPA IBA Fact sheet: Use Sites, Target Pests, and Application Methods

Use Sites: Many food and feed crops; ornamental turf and nursery plants
Uses: Growth enhancer to increase both yield and quality.
Application Methods: Applied to soil or plants as spray. Also used as a dip for cuttings.

From the US EPA IBA Fact sheet: Assessing Risks to Human Health

With the exception of certain workers, no harm is expected from use of indole-3-butyric acid. The active ingredient is not toxic to humans or other mammals.
Furthermore, indole-3-butyric acid is effective at very low concentrations--often several orders of magnitude below 1%.
It is applied at very low rates compared with most other pesticides. In animals, indole-3-butyric acid is rapidly broken down to a closely related, harmless chemical that occurs naturally in living organisms.

Eye irritation to certain workers is EPA' s only health concern for products containing indole-3-butyric acid.
For products that may cause eye irritation, workers (such as mixers and applicators) are required to use protective eye wear, such as goggles, face shield, or safety glasses.

From the US EPA IBA Fact sheet: Assessing Risks to the Environment

No risks to the environment are expected from use of this active ingredient because
1) it does not harm animals or plants in the tiny amounts used,
2) it acts as a plant growth enhancer,
3) it does not persist in the environment,
4) it is closely related to naturally occurring substances.

From the US EPA IBA Fact sheet: Regulatory Information

Products containing indole-3-butyric acid were initially registered (licensed for sale and distribution) in 1960 for use on ornamental plant cuttings and transplants.
As part of EPA' s ongoing review to ensure that pesticide products meet current standards, the chemical was reviewed and found eligible for re-registration in 1992.
As of May 2000, there were more than 40 products containing indole-3-butyric acid as an active ingredient.

From the US EPA IBA Fact sheet: Registrant Information	Many companies have registered pesticide products that contain indole-3-butyric acid. Indole-3-butyric acid enhances the growth and development of food crops and ornamentals when applied to soil, cuttings, or leaves. Because it is similar in structure to naturally occurring substances and is used in tiny amounts, this plant growth regulator poses no known risks to humans or the environment.

PROPOSED CONCLUSIONS FOR PETITION REVIEW BY THE NOSB

(1) potential of such substance for detrimental chemical interactions with other materials used in organic farming systems	None.
(2) toxicity and mode of action of the substance and of its breakdown products or other contaminants and their persistence and areas of concentration in the environment	Low toxicity for both the active ingredient and possible breakdown products. No concern for persistence and areas of concentration in the environment.
(3) probability of environmental contamination during manufacture, use, misuse or disposal of such substances	None when disposed using approved label instructions.
(4) effect of the substance on human health.	None when applied using approved personal protection equipment (PPE).
(5) effects of the substance on biological and chemical interactions in the agroecosystem ...	None. The US EPA has stipulated: "it does not persist in the environment".

(6) alternatives to using the substance in terms of practices or other materials *available*	No alternative substances are available as US EPA registered plant growth regulator substances. All plant growth regulators are required to be registered with the US EPA under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). There are no state of the art procedures using alternative substances which do not require US EPA registration.
Other verifications:	
Verification of availability of the substance	As of August 16, 2008 US EPA has this substance registered in various formulations by at least 24 companies.
(A) why the substance should be permitted in the production or handling of an organic product.	The substance has a long history to use useful by plant growers. The US EPA has stipulated: "Indole-3-butyric acid is used on many crops and ornamentals to promote growth and development of roots, flowers and fruits, and to increase crop yields. Growers find it more effective and efficient than its natural counterpart because plants cannot break it down as quickly. No harm to humans or the environment is expected to result from use of Indole-3-butyric acid."
(B) The current industry information regarding availability of and history of an organic form in the appropriate form, quality, or quantity of the substance.	(1) There are no organic forms of the substance. the US EPA has ruled that Indole-3-butyric acid, "IBA has been classified as a biochemical pesticide because it is similar in structure and function to the naturally occurring plant growth Indole-3-acetic acid" IAA. There are no US EPA registered substances which contain IAA either as a technical grade product or an end use product. Due to the mode of activity of IAA it is unlikely that manufacturers will register IAA in the future. (2) The substance is manufactured internationally; there is no possibility of loss of availability pf the substance. There are many manufacturers of the substance in the US.

(3) There are always adequate supplies of the substance in commercial trade channels.

(4) There are no trade related issues involving availability of the substance.

(5) There is a ready supply of the substance available to growers at competitive prices.

PETITION

Item A: section the petitioned substance will be included

Synthetic substances allowed for use in organic crop production, 205.601

Item B: information on the substance being petitioned

(1) substance chemical name **substance common name**	**Indole-3-butyric acid** **IBA**
(2) Manufacturer's name: **Contact person:** **Title:** **Company:** **Address:** **Email address:** **Phone:** **Fax:**	 Joel Kroin President Hortus USA Corp. 245 West 24th Street New York NY 10011 support@hortus.com 212-929-0927 212-624-0202
(3) Intended use	Plant growth regulator
(4) List of crops	Any plants which are propagated from cuttings. Any plants which can benefit from growth enhancement to increase both yield and quality. Many food and feed crops; ornamental turf and nursery plants.
(5) Source of the substance	Substance is a synthesized bio-simulator. Technical grade substance is available from many commercial sources. End use products are compounded from the technical grade substance.
(6) Previous reviews	None are known.
(7) US EPA registration numbers	Rhizopon AA #1, US EPA reg# 63310-19 Rhizopon AA #2 US EPA reg# 63310-20 Rhizopon AA #3 US EPA reg# 63310-21 Rhizopon AA Water Soluble Tablets US EPA reg# 63310-8 Hortus IBA Water Soluble Salts US EPA reg# 63310-22

(8) CAS number product labels

CAS number: 133-32-4

Product labels attached:
US EPA approved stamped product labels for:
Rhizopon AA #1,
US EPA reg# 63310-19
Rhizopon AA #2
US EPA reg# 63310-20
Rhizopon AA #3
US EPA reg# 63310-21
Rhizopon AA Water Soluble Tablets
US EPA reg# 63310-8
Hortus IBA Water Soluble Salts
US EPA reg# 63310-22

(9) substances physical properties

Rhizopon AA #1, Rhizopon AA #2, Rhizopon AA #3 and Hortus IBA Water Soluble Salts are dry powders
Rhizopon AA Water Soluble Tablets is a compounded tablet

Rhizopon AA #1, Rhizopon AA #2, Rhizopon AA #3 are insoluble in water
Hortus IBA Water Soluble Salts are Rhizopon AA Water Soluble Tablets are soluble in water

MSDS with physical properties are attached:
Rhizopon AA #1,
US EPA reg# 63310-19
Rhizopon AA #2
US EPA reg# 63310-20
Rhizopon AA #3
US EPA reg# 63310-21
Rhizopon AA Water Soluble Tablets
US EPA reg# 63310-8
Hortus IBA Water Soluble Salts
US EPA reg# 63310-22

(a) interactions with other substances used in organic production	No interactions. Rhizopon AA Water Soluble Tablets US EPA reg# 63310-8 and Hortus IBA Water Soluble Salts US EPA reg# 63310-22 can be mixed with aqueous solutions of any other substances used in organic production. Rhizopon AA #1, US EPA reg# 63310-19 Rhizopon AA #2, US EPA reg# 63310-20 Rhizopon AA #3, US EPA reg# 63310-21 are insoluble in water and will not mix with other substances used in organic production.

(b) toxicity

Toxicity is listed on the US EPA R.E.D. for IBA (attached).

Toxicity
All generic toxicology data requirements have been waived for IBA. The registered uses result in very low exposure to workers and negligible residues on crops. Products are formulated with very low percentages of IBA (0.0004 to 4.5%) and are applied at ultra-low rates (7 mg IBA/acre). Formulated products generally are of low toxicity. Additionally, IBA is metabolized to 3-indole acetic acid which is a common metabolite in tryptophan (an amino acid) metabolism in humans.

Occupational Exposure
People may be exposed to IBA during mixing, loading and application activities. However, IBA is of low toxicity and is applied at extremely low rates, so exposure data have not been required.
EPA has no significant exposure concerns other than protecting the eyes of mixers, loaders and applicators. Thus, IBA products in Toxicity Category II for primary eye irritation must bear appropriate label precautions, including a requirement that applicators wear protective eyewear (goggles, face shield, or safety glasses).

Human Risk Assessment
The Agency believes the potential risks to humans from occupational and dietary exposure to IBA are negligible.

(c) environmental impact

Environmental impact is listed on the US EPA R.E.D. for IBA (attached)

"Ecological Effects and Environmental Fate

Due to IBA's ultra-low application rates, its behavior as a plant growth hormone, and its similarity in structure and function to other naturally occurring chemicals, EPA believes that IBA poses a negligible risk to the environment."

"Environmental Risk Assessment

EPA concludes for the reasons stated above that the current uses of IBA pose a negligible risk to the environment."

(d) effects on human health

Effects on human health is listed on the US EPA R.E.D. for IBA (attached)

Toxicity

All generic toxicology data requirements have been waived for IBA. The registered uses result in very low exposure to workers and negligible residues on crops. Products are formulated with very low percentages of IBA (0.0004 to 4.5%) and are applied at ultra-low rates (7 mg IBA/acre). Formulated products generally are of low toxicity. Additionally, IBA is metabolized to 3-indole acetic acid which is a common metabolite in tryptophan (an amino acid) metabolism in humans.

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Human Risk Assessment

The Agency believes the potential risks to humans from occupational and dietary exposure to IBA are negligible.

(e) effects on soil organisms, crops or livestock

Effects on plants is listed on the US EPA R.E.D. for IBA (attached)

Use profile:
IBA is a plant growth regulator, used to promote and accelerate root formation of plant clippings and to reduce transplant shock of non-food ornamental nursery stock. IBA is also used on fruit and vegetable crops, field crops and ornamental turf to promote growth development of flowers and fruit and to increase crop yields. IBA has been classified as a biochemical pesticide because it is similar in structure and function to the naturally-occurring plant growth hormone indole-3-acetic acid.

(10) Safety information

(1) MSDS with physical properties and safety information are attached:
Rhizopon AA #1,
US EPA reg# 63310-19
Rhizopon AA #2
US EPA reg# 63310-20
Rhizopon AA #3
US EPA reg# 63310-21
Rhizopon AA Water Soluble Tablets
US EPA reg# 63310-8
Hortus IBA Water Soluble Salts
US EPA reg# 63310-22

(2) Copies of the US EPA stamped labels for US EPA Registered products are attached.
Rhizopon AA #1,
US EPA reg# 63310-19
Rhizopon AA #2
US EPA reg# 63310-20
Rhizopon AA #3
US EPA reg# 63310-21
Rhizopon AA Water Soluble Tablets
US EPA reg# 63310-8
Hortus IBA Water Soluble Salts
US EPA reg# 63310-22

These labels were written for safety information by the US EPA in compliance with the US Department of Labor Worker Protection Safety Act. WPS

(3) Search of the the National Toxicology Program web site
http://ntp-apps.niehs.nih.gov/ntp_tox/index.cfm for the CAS 133-32-4:

"Search found
Found 0 Search Result for Search Term '133-32-4'
No chemical(s) were found based on this search term."

(11) Research information

Attached are selected chapters from:
Peter J. Davies, editor, Plant Hormones, Biosynthesis, Signal Translocation, Action! Kluwer Academic Publishers, Dordrecht, the Netherlands. 2004
(1) The plant hormones: their nature, occurrence and function. Peter J. Davies
(2) Auxin biosynthesis and metabolism. J. Normaly, J. Slovin, J. Cohen

Tim D. Davis, Bruce E. Haisig and Narendra Sankhla. editors. Adventitious root formation in cuttings. Advances in Plant Sciences Series. Volume 2. Dioscorides Press. Portland OR. 1988
(1) Chapter 9. Auxin metabolism during adventitious rooting. Thomas Gaspar and Michel Hofinger
(2) Chapter 10. Chemicals and formulations used to promote adventitious rooting. Frank A. Blazich

(12) Petition justification statement

(a) why the synthetic substance is necessary for the production or handling of an organic product.

Plants can be reproduced in various ways including from seed, from grafts, and from cuttings. Seeds often do not reproduce the next generation as the parent plant. In order to maintain genetic integrity clonal propagation produces new plants that are identical to the parent plant. Plant propagation from cuttings assures that the new generation of plants is identical to the parent plant. While plants generate natural root producing substances, auxins, these substances are not adequate for consistent rooting. Also, many plant varieties are not possible to reproduce from cuttings unless root inducing substances are applied. Indole-3-butyric acid is the most useful rooting inducing substance.

Indole-3-butyric acid is also useful to enhance the root formation in plants that are starting to root but need additional applied root inducing substances to form uniform root systems.

(b) substances on the National List or other cultural methods that could be used in place of the petitioned synthetic substance.

No other substances on the National List are suitable as root inducing substances.

Historically, plant growers for centuries had tried to find root inducing substances that are useful when propagating new plants and to improve plant growth. In the 1930's the natural root inducing substance, IAA, was discovered. When the chemical composition of IAA was identified then other root inducing substances were developed based upon the structure of IAA. Indole-3-butyric acid has been the most successful substance used as a root inducing substance since it is very stable and it is controlled and regulated by the plant. It is useful for root inducing on most plant varieties which can be propagated from cuttings.

(c) beneficial effects to the environment, human health , or farm ecosystem from use of the synthetic substance that support its use instead of the use of a non-synthetic substance or alternative cultural method.

(1) Indole-3-butyric acid has been found to have no hazardous effects to the environment.

The US EPA R.E.D. for IBA (attached).
The US EPA states:
"Ecological Effects and Environmental Fate
Due to IBA's ultra-low application rates, its behavior as a plant growth hormone, and its similarity in structure and function to other naturally occurring chemicals, EPA believes that IBA poses a negligible risk to the environment."

(2) Indole-3-butyric acid has been shown to be non-hazardous to humans when used in accordance to standard personal protection procedures.

The US EPA R.E.D. for IBA (attached).
The US EPA states:
"Human Risk Assessment.
The Agency believes the potential risks to humans from occupational and dietary exposure to IBA are negligible."

(3) There are no other non-synthetic substances or alternative cultural methods available.

ATTACHMENTS

Peter J. Davies, editor. Plant Hormones, Biosynthesis, Signal Translocation, Action!
Kluwer Academic Publishers, Dordrecht, the Netherlands. 2004
(1) The plant hormones: their nature, occurrence and function. Peter J. Davies
(2) Auxin biosynthesis and metabolism. J. Normaly, J. Slovin, J. Cohen

Tim D. Davis, Bruce E. Haisig and Narendra Sankhla. editors.
Adventitious root formation in cuttings.
Advances in Plant Sciences Series. Volume 2. Dioscorides Press. Portland OR. 1988
(1) Chapter 9. Auxin metabolism during adventitious rooting. Thomas Gaspar and Michel Hofinger
(2) Chapter 10. Chemicals and formulations used to promote adventitious rooting. Frank A. Blazich

MSDS with physical properties and safety:
- Rhizopon AA #1, US EPA reg# 63310-19
- Rhizopon AA #2, US EPA reg# 63310-20
- Rhizopon AA #3, US EPA reg# 63310-21
- Rhizopon AA Water Soluble Tablets, US EPA reg# 63310-8
- Hortus IBA Water Soluble Salts, US EPA reg# 63310-22

US EPA stamped approval labels:
- Rhizopon AA #1, US EPA reg# 63310-19
- Rhizopon AA #2, US EPA reg# 63310-20
- Rhizopon AA #3, US EPA reg# 63310-21
- Rhizopon AA Water Soluble Tablets, US EPA reg# 63310-8
- Hortus IBA Water Soluble Salts, US EPA reg# 63310-22

- US EPA R.E.D Facts: Indole-3-butyric acid. August 1992
- US EPA R.E.D Eligibility Requirements: Indole-3-butyric acid. August 1992
- US EPA FIFRA: definitions and registration requirements
- US EPA registered IBA containing products by product name: 2008
- US EPA registrants of IBA containing products: 2008
- US EPA registered active ingredients, alphabetical list 2008