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24-EPIBRASSINOLIDE
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CA 8 FATE AND BEHAVIOUR IN THE ENVIRONMENT

This document presents studies on the fate and behaviour in the environment of the active substance 24-Epibrassinolide.

For the inclusion of the active substance 24-Epibrassinolide and the representative formulation Sunergist (0.01 % 24-Epibrassinolide) in Annex I, data to support the application for inclusion is provided in the following section. Studies, where available, are summarised under the respective data points. In some cases, public literature is used to address data points. In the case where published literature is used to address a data point, an extended summary of the published literature is provided and cited.

In the case where published literature is used to scientifically justify why a study was not deemed necessary to be conducted or as supporting information, only authors and year is given in the text, while full bibliographical information can be found in "Annex I: Publications to support evaluation" at the end of each section. Relevant literature from the EFSA- compliant literature search, which has to be evaluated on full-text level, is discussed under the respective data point.

The applicant submitted an extensive introduction to brassinosteroids in support with the dossier of 24-Epibrassinolide. This general information was not evaluated in detail by RMS because it is not deemed necessary for the DAR preparation but is provided in Appendix I for completeness. Nonetheless a short version was extracted and accepted by RMS and is presented below to give an overview.

CA 8.0 General Introduction

General Introduction

Brassinosteroids, including 24-Epibrassinolide are naturally occurring, plant growth promoting molecules, present in higher plants, lower plants, including algae, mosses, the "living fossil" *Equisetum* as well as some fungi.[1,2,3] Brassinosteroids are present in all plant organs such as pollen, anthers, seeds, leaves, stems, roots, flowers, grains and fruits with the highest concentrations found in pollen, seeds and fruits and considered an obligatory plant constituent.[4,5]
Brassinosteroids are essential for normal plant growth and development. Those phylogenetically ancient phytohormones, evolved in the Pre-Cambrian, it can be expected that each organism has developed its own co-evolutionary mechanism to metabolise these phytohormones.[6] 24-Epibrassinolide elicits and activates the plant's self-defence mechanisms mediating the plant's resistance to unfavourable

[1] KCA 8/0001: Takatsuto, S., Abe, H., Gamoah, K. (1990): EVIDENCE FOR BRASSINOSTEROIDS IN STROBILUS OF EQUISETUM ARVENSE L. Report No.: na (092-059) Agricultural and Biological Chemistry, 1990, 54 (4), 1057-1059; Not GLP, published

[2] KCA 8/0011: Bajguz, A., Tretyn, A. (2003): THE CHEMICAL STRUCTURES AND OCCURRENCE OF BRASSINOSTEROIDS IN PLANTS. Report No.: na (092-145). Brassinosteroids. Chapter 1, 2003, 1-44. Not GLP, published.

[3] KCA 8/0012: Bajguz, A. (2011): BRASSINOSTEROIDS – OCCURRENCE AND CHEMICAL STRUCTURES IN PLANTS. In: Hayat, S., Ahmad, A.: BRASSINOSTEROIDS: A CLASS OF PLANT HORMONE. Report No.: na (092-146). Springer Verlag, 2011, Chapter 1, 1-27, DOI 10.1007/978-94-007-0189-2_1; ISBN: 978-94-007-0188-5. Not GLP, published

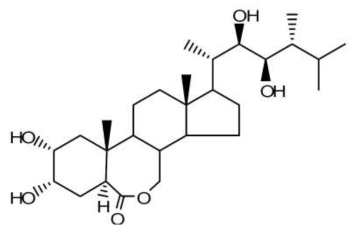
[4] KCA 8/0002: Zhu, J.-Y., Sae-Seaw, J., Wang, Z.-Y. (2013): BRASSINOSTEROID SIGNALLING. Report No.: na (092-165). Development, 2013, 140(8), 1615-1620; doi: 10.1242/dev.060590. Not GLP, published.

[5] KCA 8/0012: Codreanu, M.; Russinova, E. (2011): REGULATORY MECHANISMS OF BRASSINOSTEROID SIGNALING IN PLANTS. In: Hayat, S., Ahmad, A. (eds.): BRASSINOSTEROIDS: A CLASS OF PLANT HORMONE. Report No.: na (092-146). Springer Verlag, 2011, Chapter 2, 29-56, DOI 10.1007/978-94-007-0189-2_2; ISBN: 978-94-007-0188-5. Not GLP, published

[6] KCA 8/0005: Kutschera, U., Wang, Z.-Y. (2012): BRASSINOSTEROID ACTION IN FLOWERING PLANTS: A DARWINIAN PERSPECTIVE. Report No.: na (092-036). Journal of Experimental Botany, 2012, 63 (10), 3511-3522; doi:10.1093/jxb/ers065. Not GLP, published

environmental conditions, (e.g. salinity, drought, cold and heat stress) and fungal diseases.[7] Application of brassinosteroids leads to a complex sequence of biochemical reactions such as activation or suppression of key enzymatic reactions, induction of protein synthesis and the production of various chemical defence compounds.[8]

Table 8.0-1 : Substances and metabolites of environmental relevance (structure, synonyms and codes)

Code	IUPAC name	Compound found in	Structural formula
24-Epibrassinolide	(22R,23R,24R)-2α,3α,22,23-tetrahydroxy-24-methyl-β-homo-7-oxa-5-cholestan-6-one	Environment (soil, surface water), plant, rat	
No relevant metabolites. Due to the natural occurrence of 24-Epibrassinolide and its metabolites, risk assessments for metabolites are not considered necessary as they are deemed to be covered by the parent.			

CA 8.1 Fate and Behaviour in Soil

CA 8.1.1 Route of degradation in soil

CA 8.1.1.1 Aerobic degradation

Brassinosteroids, including 24-Epibrassinolide are naturally occurring phylogenetically ancient phytohormones, found throughout the plant kingdom as well as some fungi. 24-Epibrassinolide elicits and activates the plant’s self-defence mechanisms meditating the plant’s resistance to unfavourable environmental factors, stress and diseases.

Due to the constant formation and decomposition of plant root systems, the presence of seeds, pollen, and decomposing plant material and the release of Brassinosteroids from decomposing organic matter (e.g. Aremu *et al*., 2015) as well as the vast number of other Brassinosteroid producing organisms such as algae in the environment, similar as for all other phytosterols, Brassinosteroids are expected to be naturally present in all environmental compartments including soil, water-bodies and sediment (Aremu *et al*., 2015, Hassett and Lee, 1977; Mudge *et al*. 1999).

In 1942, Turfitt (1942) analysed the sterol contents of British soils. Samples were taken from different vegetative types and soil types. Sterol concentrations between 0 (acid sand or loam from grasslands) and 12.7 mg/kg soil (from deep acid peat taken from peat and spring moors) were measured. More recently, Heumann *et al*. (2011) studied the phytosterol content in soil samples. The samples were taken from different soil types such as podzoles, gleysoles cambisols and intermediates. The soil sterol concentrations ranged between 100 and 3600 mg/kg soil.

[7] KCA 8/0012: Kang, Y., Guo. S. (2011): ROLE OF BRASSINOSEROIDS ON HORTICULTURAL CROPS. In: Hayat, S., Ahmad, A. (eds.): BRASSINOSTEROIDS: A CLASS OF PLANT HORMONE. Report No.: na (092-146). Springer Verlag, 2011, Chapter 9, 269-288, DOI 10.1007/978-94-007-0189-2_9; ISBN: 978-94-007-0188-5. Not GLP, published

[8] KCA 8/0091: Bajguz, A., Hayat, S. (2009): EFFECTS OF BRASSINOSTEROIDS ON THE PLANT RESPONSES TO ENVIRONMENTAL STRESSES. Report No.: na (092-133). Plant Physiology and Biochemistry, 2009, 47, 1-8; doi:10.1016/j.plaphy.2008.10.002. Not GLP, published

No information on the exact concentrations of Brassinosteroids in different soil types are available. For vermicompost, Aremu *et al.*, 2015 measured Brassinosteroid concentrations between 3.084 ng and 3.809 ng per litre of vermicompost leachate (fg/mL).

The degradation pathways of 24-Epibrassinolide and Brassinosteroids in soil and other environmental compartments have not yet been investigated completely. Nevertheless, some studies have been performed on the metabolic pathway in plants and fungi. There is no uniform degradation pathway but different plant and fungal species or groups have their own pathways. As Brassinosteroids are phylogenetically ancient phytohormones, evolved in the Pre-Cambrian (Kutschera and Wang, 2012), the development of various co-evolutionary organism- and matrix-specific mechanisms for degradation, metabolism and catabolism of this phytohormone can be expected.

The plant metabolism process of 24-Epibrassinolide includes hydrogenation, hydroxylation, esterification and glycosylation (Table 7.1.1.1-1; Bajguz *et al.*, 2007; Fujioka and Yokota, 2003). The metabolism pathway is not completely known and strongly depends on the plant species and plant organ. In tomato cells, the major metabolites of 24-epibrasinolide are 25-Hydroxy-24-epi-brassinolide and 6-Hydroxy-24-epi-brassinolide that is further metabolised to 25-β-D-glucosyloxy-24-epiepibrasinolide and 25-hydroxy-24-Epibrassinolide (Hai *et al.*, 1995; Schneider *et al.*, 1994). In seradella (*O. sativus)* cells, 24-Epibrassinolide is converted to 3,24- diepibrasinolide which is further metabolised to a mixture of 3-laurate, 3-myristate and 3-palmitate (Kolbe *et al.*, 1995). In cucumber, 24-epiBl is metabolized to 2,24-diepibrassinolide in the second leaves and petioles, but not in hypocotyls and roots (Nishikawa *et al.*, 1995).

Table 8.1.1.1-1: Metabolic reactions of 24-Epibrassinolide in plants (Fujioka and Yokota, 2003; Bajguz *et al.*, 2007)

Reaction	**Site**	**Substrate**	**Product**	**Plant**	**Reference**
Hydrogenation	2α-OH	24-Epibrassinolide	2,24-diepibrassinolide	*Cucumber (C. sativus)*	Nishikawa *et al.*, 1995
	3αOH	24-Epibrassinolide	3,24-diepi diepibrassinolide	Serradella (*O. sativus)*	Kolbe *et al.*, 1996
Hydroxylation	C-12	24-Epibrassinolide	12β-hydroxy-24-Epibrassinolide	*Fungi (C. echinulata)*	Voigt *et al.*, 1993
	C-20	3,24-diepbrassinolide	20-hydroxy-3,24-diepibrassinolide	Serradella (*O. sativus)*	Kolbe *et al.*, 1996
	C-25	24-Epibrassinolide	25-hydroxy-24-Epibrassinolide	*Tomato (L. esculentum)*	Winter *et al.*, 1997 Hai *et al.*, 1995 Schneider *et al.*, 1994
	C-25	3,24-diepibrassinolide	25-hydroxy-3,24-diepibrassinolide	Serradella (*O. sativus)*	Kolbe *et al.*, 1996
	C-26	24-Epibrassinolide	26-hydroxy-24-Epibrassinolide	*Tomato (L. esculentum)*	Winter *et al.*, 1997 Hai *et al.*, 1995 Schneider *et al.*, 1994
Side chain cleavage	C-20/22	3,24-diepibrassinolide	3,24-diepibrassinolide C_{21}-catabolite	Serradella (*O. sativus)*	Kolbe *et al.*, 1996
Esterification	3β-OH	3,24-diepibrassinolide	3,24-diepibrassinolide-3-palmitate ($R = C_{15}H_{31}$) 3,24-diepibrassinolide-3-myristate ($R = C_{13}H_{27}$) 3,24-diepibrassinolide-3-laurate ($R = C_{11}H_{23}$)	Serradella (*O. sativus)*	Kolbe *et al.*, 1995
Glycosylation	25-OH	25-hydroxy-24-Epibrassinolide	25-hydroxy-24-Epibrassinolide-25-*O*-β-	*Tomato (L. esculentum)*	Winter *et al.*, 1997 Hai *et al.*, 1995

Reaction	Site	Substrate	Product	Plant	Reference
			glucoside		Schneider *et al*., 1994
	26-OH	26-hydroxy-24-epibrassinolide	26-hydroxy-24-Epibrassinolide-26-*O*-b-glucoside	*Tomato (L. esculentum)*	Winter *et al*., 1997 Hai *et al*., 1995 Schneider *et al*., 1994

It is also known that Brassinosteroids are metabolized by certain microorganisms. For example, 24-Epicastasterone and 24-Epibrassinolide are transformed by the fungus *Cunninghamella echinulata* to give the corresponding 12β-hydroxylated compounds (eg. 12β-hydroxy-24-Epibrassinolide) (Voigt *et al*., 1993).

Furthermore, although the complete metabolic pathway of 24-Epibrassinolide in soil is not yet known, no different metabolic/degradation pathways of the 'natural-identical synthesized molecule' 24-Epibrassinolide to be used for agricultural purposes compared to the natural occurring 24-Epibrassinolide are to be expected. As Brassinosteroids are phylogenetically ancient phytohormones, evolved in the Pre-Cambrian, the development of various co-evolutionary matrix-specific mechanisms for degradation of this phytohormone can be expected.

In addition to that, for agricultural purposes, only low amounts of the natural-identical synthesized molecule, 24-Epibrassinolide, are used and thus the artificial release will influence natural background levels only to a limited extend. This is for example highlighted by Khripach *et al.* (2000). For typical quantities of Brassinosteroids of 5-50 mg per hectare used in agriculture the authors calculate for the highest dosage an average Brassinosteroid concentration of 2.1 x 10^{-3} nmol/g plant biomass assuming a total weight of 50 tons of biomass per hectare and full absorption by plants. The authors conclude that this is close to the natural Brassinosteroids concentration in plants. This is also compliant the proposed application rates for the representative formulation (please refer to Document D3).

As known from several studies (please refer to MCA 6) and as evident from studies on the plant strengthening mode of action of vermicompost (Aremu *et al*., 2015) free phytohormones such as Brassinosteroids in soil are readily taken up by plants. Thus, it is to be expected that in case Brassinosteroids occur freely in soil, e.g. if Brassinosteroids are released by degradation of organic plant matter (Aremu *et al*., 2015) or during the use of Brassinosteroid-containing plant protection products, Brassinosteroids are taken up by roots and subsequently metabolised by plants.

Comment RMS:

RMS has revised the open public literature refered in this point. Though little is known from the exact degradation pathway of brassinosteroids in soil, the Notifier provides information on the natural existence of the substances in the environment and on possible degradation pathways. Brassinosteroids are phylogenetically ancient phytohormones, evolved in the Pre-Cambrian, and the development of various co-evolutionary matrix-specific mechanisms for degradation of this phytohormone can be expected. Uptake, immobilisation, metabolism and release of brassinosteroids are expected from plants, algea, fungi and bacterias.

No different metabolic/degradation pathways are expected between the natural occurring 24-Epibrassinolide and the synthesized molecule 24-Epibrassinolide to be used for agricultural purposes.

CA 8.1.1.2 Anaerobic degradation

For the intended uses the exposure of 24-Epibrassinolide to anaerobic conditions is unlikely to occur and even if it does occur, no difference to the degradation of natural 24-Epibrassinolide is expected.

CA 8.1.1.3 Soil photolysis

Photolysis is not expected to contribute significantly to the degradation of 24-Epibrassinolide due to the low light absorbance of the active substance at a wavelength of 295 nm (please refer to MCA 2).

CA 8.1.2 Rate of Degradation in Soil

CA 8.1.2.1 Laboratory studies

CA 8.1.2.1.1 Aerobic degradation of the active substance

Data point addressed:	CA 8.1.2.1.1/01
Author(s) (year):	Chen, S., Shi, L., Shan, Z., Hu, Q. (2005)
Title:	CHARACTERISTICS OF HYDROLYSIS AND DEGRADATION OF BRASSINOLIDE IN SOILS
Laboratory report / project Number (Doc. No.):	Not applicable (092-001)
Testing facility:	Not applicable
Published:	Yes (Rural Eco-Environment, 2005, 21 (1), 55-57)
Test guideline used:	Not indicated
Deviations:	None
GLP:	No

Executive Summary

Hydrolysis characteristics of Brassinolide at different pH values and temperatures and its degradation characteristics in four soils, including Northeast China black soil, sterilized Northeast China black soil, Jiangxi red soil, and Henan fluvo-aquic soil, were investigated. In the following, the aerobic degradation in is described. The hydrolysis properties researched by the authors are described under CA 8.2.1.1.

Results of the aerobic degradation study show that Brassinolide degradation follows first-order kinetics. The degradation half-life at 25 °C was 13.8 days in Northeast China black soil, 14.1 days in sterilized Northeast China black soil, 16.5 days in Henan fluvo-aquic soil and 43.3 days in Jiangxi red soil respectively.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:
Brassinolide reference standard with a mass fraction greater than 99% was supplied by Yunda Group.

2. Soil:
Jiangxi red soil, Henan fluvo-aquic soil and Northeast China black soil were air-dried and sifted through a sieve with pore size of 0.84 mm (20 meshes) before use. The physical and chemical properties of the test soils are shown in Table 7.1.2.1.1-1.

Table 8.1.2.1.1-1: Physical and chemical properties of test soils

Type	Characteristics	pH value	Organic matter (g/kg)	Cation exchange capacity (cmol/kg)
Jiangxi red soil	Loamy clay	5.29	9.94	10. 60
Henan fluvo-aquic	Loam	9.17	16.90	8.46

soil				
Northeast China black soil	Clay	8.45	28.70	29. 80

3. Instruments and apparatus

Waters HPLC system, Waters 486 ultraviolet detector, rotary evaporator, autoclave (Tomy, Japan), thermostatic incubator, high-speed centrifuge.

B. STUDY DESIGN

1. Experimental conditions

20.0 g accurately weighed Northeast China black soil, Jiangxi red soil and Henan fluvo-aquic soil were respectively transferred into 3 groups of 100 mL Erlenmeyer flasks (each group included 10 flasks). A certain amount of Brassinolide solution was added into each Erlenmeyer flask, and the water content in the soil was adjusted to 60% of its maximum water-holding capacity. All Erlenmeyer flasks were stoppered with cotton plugs, placed into 25 ℃ thermostatic incubator, and samples were collected periodically for determining the residual Brassinolide concentration in soil. An additional group of samples were prepared using autoclaved Northeast China black soil.

2. Extraction

For the extraction of Brassinolide from soil, (50+50) mL acetone was added into each soil sample, shaken for 1.0h at 25 °C, and separated by high-speed centrifugation. Extracts were combined, subjected to rotary evaporation to remove acetone, and 50 mL deionized water added. The extracts were mixed evenly by shaking and transferred to a 125mL separatory funnel.

3. Analytical conditions

The chromatographic conditions were: Nucleosil 100 - 5 C8 stainless steel column (250 mm×4. 6 mm); mobile phase: V(acetonitrile): V(water)= 75:25; flow rate: 1 mL/min; column temperature: 37°C; detection wavelength: UV 222nm; sensitivity: 0.2 AUFS. Brassinolide had a retention time (tR) of 5.6 min under the aforesaid chromatographic conditions.

The minimum detectable amount of Brassinolide was 2×10^{-9} g. For the determination of recovery rate, 0.5 - 10 mg Brassinolide per kg of soil was added. The recovery rate of Brassinolide was 84.3-93.2 %.

II. RESULTS AND DISCUSSION

The degradation characteristics of Brassinolide in Northeast China black soil, Henan fluvo-aquic soil and Jiangxi red soil were determined at 25°C. The test results are shown in Figure 8.1.2.1.1-1 and Table 8.1.2.1.1-2.

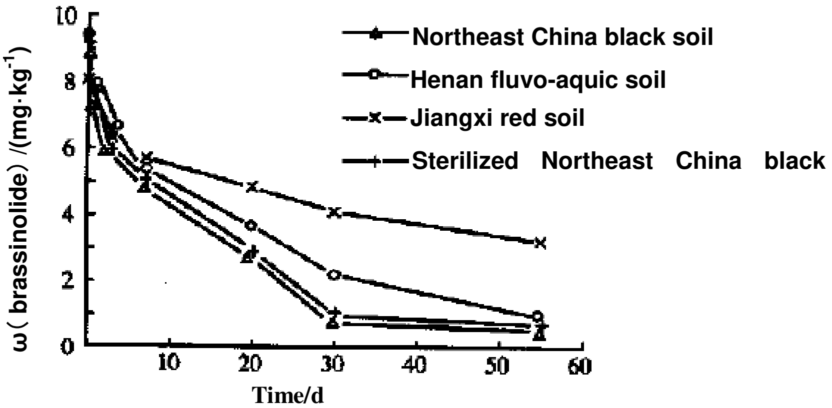


Figure 8.1.2.1.1-1: Degradation of Brassinolide in four soils at 25 °C

Table 8.1.2.1.1-2: Kinetic parameters of the degradation of Brassinolide in four different soils

Type	Degradation rate, d^{-1}	Half-life, d
Northeast China black soil	0.050	13.8
Sterilized Northeast China black soil	0.049	14.1
Henan fluvo-aquic soil	0.042	16.5
Jiangxi red soil	0.016	43.3

As shown in Figure 7.1.2.1.1-1 and Table 7.1.2.1.1-2, the degradation of Brassinolide in three test soils followed the first-order kinetics. The degradation half-life for Northeast China black soil, Henan fluvo-aquic soil, Jiangxi red soil was 13.8, 16.5 and 43.3 days respectively.

The degradation of Brassinolide was affected by organic matter in soil and pH value. Brassinolide degraded quickly in highly alkaline Northeast China black soil with a high organic matter content, and slower in acidic red soil with a low organic matter content.

It had a degradation half-life of less than 30 days both in Northeast China black soil and Henan fluvo-aquic soil as an easily degradable substance; whereas it is a moderately degradable substance in Jiangxi red soil (less than 60 days).

The degradation characteristics of Brassinolide in sterilized and unsterilized soils were also determined. Results show that Brassinolide had a degradation half-life of 14.1 days in sterilized Northeast China black soil, indicating that sterilization imposed little effect on soil degradation. It is considered that the degradation of brassinolide in soil is mainly due to chemical degradation, and micro-biological degradation is secondary.

III. CONCLUSION

The degradation of Brassinolide followed the first-order kinetics. The degradation of Brassinolide in soil is mainly due to chemical degradation and is influenced by ph and organic matter content. According to the study, the lower the organic matter content and pH, the longer it takes for Brassinosteroids to degrade. The degradation is further supplemented with microbiological degradation.

According to the authors, Brassinolide had a degradation half-life of less than 30 days in highly alkaline Northeast China black soil and Henan fluvo-aquic soil, as an easily degradable substance, whereas it is a moderately degradable substance (less than 60 days) in acidic Jiangxi red soil.

The soil types and characteristics of the three soil types described in the study of Chen *et al*. 2005 are comparable to and representative for soils all around the world, as described below:

Northeast China black soil: According to Xingwu *et al*. (2010), the Chinese black soil belongs to Isohumisols in the Chinese Soil Taxonomy (CST) or Mollisols in the US Soil Taxonomy. Other commonly used international names are Phaeozems, Brunizems (Argentina, France), Parabraunerde-Tsjernozems (Germany) and Aquolls (Soil Atlas of Europe, 2005) whereat Aquolls are a suborder of the order of Mollisols. The Soil Atlas of Europe (2005) characterizes Phaeozems as soils that "are found in wet steppe (prairie) regions and are much like Chernozems and Kastanozems but more intensively leached in wet seasons. Consequently, they have a dark, humus-rich surface horizon and have no secondary carbonates in the upper meter of soil. Chernozems and Phaeozems are highly productive soil types and are used mainly for cereal crop production" and cover about 3 % of Europe.

Henan fluvo-aquic soils belong to the soil order of Inceptisols according to the USDA soil taxonomy system (Kong *et al*., 2014) whereat the USDA Soil Taxonomy classifies Cambisols as Inceptisols (Soil Atlas of Europe, 2005). Cambisols are young soils and "occur in a wide variety of environments around the world and under many kinds of vegetation. Commonly referred to as brown soil, Braunerde

(Germany), Sols bruns (France) or Brunizems (Russia)" and cover about 12 % of Europe (Soil Atlas of Europe, 2005).

Jiangxi red soil: According to Zhang *et al*. (2004) Chinese red soils consist of Ultisols and partly Alfisols. Ultisols and Alfisols are soil orders in the USDA soil taxonomy. According to the Soil Atlas of Europe (2005) red soils are similar to Acrisols and are related to several subgroups of Alfisols and Ultisols whereat the Soil Atlas of Europe (2005) specifies an Acrisol as "a highly weathered soil occurring in warm temperate regions and the wetter parts of the tropics and subtropics. Acrisols have poor chemical properties, low levels of plant nutrients, high levels of aluminium and high susceptibility to erosion. These conditions are strong limitations for agricultural use." Acrisols cover about 1% of Europe. Several suborders of Alfisols are closely related to other soil types or synonymous for example to Albeluvisols, Podzoluvisols (FAO) or Orthopodzolic soil (Russia). Referring to the Soil Atlas of Europe (2005) for example_"Albeluvisols occur mainly in the moist and cool temperate regions" covering about 15% of Europe and thus comprise the most common soil. Alfisol is also a synonym for Luvisols whereat Livisols "show marked textural differences within the profile. The surface horizon is depleted in clay while the subsurface 'argic' horizon has accumulated clay. A wide range of parent materials and environmental conditions lead to a great diversity of soils in this Reference Soil Group. Other names used for this soil type include Pseudo-podzolic soil (Russia), sols lessivés (France) or Parabraunerde (Germany). Luvisols cover about 6% of Europe.

Comment RMS:

The degradation study from Chen *el al.* was not performed according the OECD guideline 307. Only three soils were investigated instead of the requested four soils. No biometer-type flasks or flow-through systems were used to monitor potential volatile products. Furthermore, there is no information on whether the flasks were incubated in the dark or in the presence of light. The recovery of brassinolide in soil was low to acceptable (84.3 – 93.2%).

Degradation rates were derived from the data available at 25 °C. RMS normalised the degradation rates to 20 °C and the rates are presented in the table below.

Type	**Degradation rate at [d^{-1}] (25 °C)**	**Half-life [d] (25 °C)**	**Normalised DT_{50} [d] (20 °C)**
Northeast China black soil	0.050	13.8	22.17
Sterilized Northeast China black soil	*0.049*	*14.1*	*22.65*
Henan fluvo-aquic soil	0.042	16.5	26.50
Jiangxi red soil	0.016	43.3	69.55
Geometric mean (n=3)		**21.44**	**34.44**

The degradation rate of the sterilized northeast China black soil was not taken into consideration in the calculation of the geometric mean. As only three values are available, and due to the flaws of the study, RMS considers the values to be of informative quality and the geometric mean not to be of sufficient strength to be used in modelling. The use of the worst case value of **69.55 days** from the Jiangxi red soil was chosen by the RMS for further modelling.

CA 8.1.2.1.2 Aerobic degradation of metabolites, breakdown and reaction products

No studies on metabolites or breakdown reaction products are necessary as 24-Epibrassinolide is naturally occurring in the environment.

CA 8.1.2.1.3 Anaerobic degradation of the active substance

For the intended uses the exposure of 24-Epibrassinolide to anaerobic conditions is unlikely to occur and even if it does occur, no difference to the degradation of natural 24-Epibrassinolide, naturally present under anaerobic conditions, is expected.

CA 8.1.2.1.4 Anaerobic degradation of metabolites, breakdown and reaction products

For the intended uses the exposure of 24-Epibrassinolide to anaerobic conditions is unlikely to occur and even if it does occur, no difference to the degradation of natural 24-Epibrassinolide is expected.

CA 8.1.2.2 Field Studies

Due to the constant formation and decomposition of plant root systems, the presence of seeds, pollen, and decomposing plant material and the release of Brassinosteroids from decomposing organic matter (e.g. Aremu *et al*., 2015) as well as the vast number of other Brassinosteroid producing organisms such as algae in the environment, Brassinosteroids are expected to be naturally present in all environmental compartments including soil and water-bodies including sediment (Aremu *et al*., 2015, Hassett and Lee, 1977; Mudge *et al*. 1999), as is the case for all natural phytosterols.

No field studies are therefore considered necessary.

Comment RMS:
Though the RMS considers DT_{50} in soil to be 69.55 days, it does not consider explicit field studies to be necessary for the assessment. This is based on the ubiquitous presence of brassinosteroids in the environment and the constant formation and decomposition of the substances.

CA 8.1.2.2.1 Soil dissipation studies

Not required. Please see CA 8.1.2.2 above.

CA 8.1.2.2.2 Soil accumulation studies

Not required. Please see CA 8.1.2.2 above.

CA 8.1.3 Absorption and desorption in soil

CA 8.1.3.1 Adsorption and desorption

Due to the constant formation and decomposition of plant root systems, the presence of seeds, pollen, and decomposing plant material and the release of Brassinosteroids from decomposing organic matter (e.g. Aremu *et al*., 2015) as well as the vast number of other Brassinosteroid producing organisms such as algae in the environment, Brassinosteroids are expected to be naturally present in all environmental compartments including soil and water-bodies as well as sediment (Aremu *et al*., 2015, Hassett and Lee, 1977; Mudge *et al*. 1999). No different adsorption or desorption of the natural-identical synthesized molecule, 24-Epibrassinolide, to the natural occurring 24-Epibrassinolide, is expected.

An important chemical property of a substance that affects its movement in soil is hydrophobicity. Hydrophobicity is usually expressed as the octanol-water partition coefficient (log Pow), where a high log

Pow value corresponds to high hydrophobicity (Briggs *et al*., 1982; Ryan *et al*., 1988). Thus, substances with lipophilicity close to that of plant roots, can be transferred from soil or water to the plant spontaneously by a diffusion-driven process. Organic compounds with log Pow ranging from 1 and 3 (moderately hydrophobic) are mostly taken up by plants (Briggs *et al*., 1982).

Furthermore, 24-Epibrassinolide is a moderately hydrophobic organic compound with a log Pow of 2.0 (please refer to MCA 2) and will be spontaneously transferred from soil or water to the plant by a diffusion-driven process and therefore not available for leaching to the different water bodies and systems. This was also confirmed by feeding experiments performed by Yokota *et al*. (1992) and Nishikawa *et al*. (1994). The authors showed that labelled Brassinosteroids was passively taken up by the roots and subsequently catabolised.

RMS Comments:
No valid information is available for the determination of the adsorption/desorption capacities of 24-Epibrassionolide. Indications on the possible behaviour of the substance in soil are provided based on the log P_{OW} of the substance. For modelling, the Notifier proposes to use the value of 0 L/kg as a worst-case K_{fOC} value. The RMS agrees to this approach for the groundwater modelling, but proposes also to perform a second set of modelling using a worst-case default K_{fOC} value of 10000 L/kg to evaluate the possible binding of the active substance to the sediment compartment.

CA 8.1.3.1.1 Adsorption and desorption of the active substance

Study not considered necessary. Please see CA 8.1.3.1 above.

CA 8.1.3.1.2 Adsorption and desorption of metabolites, breakdown and reaction products

Study not considered necessary. Please see CA 8.1.3.1 above.

CA 8.1.3.2 Aged sorption

An aged sorption study is an optional higher tier study. In view of the ubiquitous distribution of Brassinosteroids due to the natural occurrence and the low leaching potential of 24-epibrasssinolide, respective data is not considered necessary.

CA 8.1.4 Mobility in soil

Brassinosteroids are ubiquitous in the environment, and naturally present in higher plants, lower plants, including algae and mosses and in certain fungi. Brassinosteroids are present in all plant organs such as pollens, anthers, seeds, leaves, stems, roots, flowers, grains and fruits with the highest concentrations found in pollen, seeds and fruits (Zhu *et al*., 2013) and are considered obligatory plant constituents. The concentration of Brassinosteroids in plants is regulated by a complex system of feedback pathways and Brassinosteroids are constantly synthesised, metabolised, catabolised, activated and inactivated depending on the plant's needs as well as environmental cues. The concentrations of Brassinosteroids are continuously fluctuating - spatially and temporally: in a single plant, different concentrations can be measured simultaneously in different plant organs, cell structures and cells as well as in the same location at different times.

Due to the constant formation and decomposition of plant root systems, the presence of seeds, pollen, and other decomposing plant material and the release of Brassinosteroids from decomposing organic matter (e.g. Aremu *et al*., 2015) as well as the vast number of other Brassinosteroid producing organisms such as algae in the environment, Brassinosteroids are expected to be naturally present in all environmental compartments including soil and water-bodies as well as sediment (Aremu *et al*., 2015, Hassett and Lee, 1977; Mudge *et al*. 1999). No different mobility of the natural-identical synthesized molecule, 24-Epibrassinolide, to the natural occurring 24-Epibrassinolide, is expected.

An important chemical property of a substance that affects its movement in soil is hydrophobicity. Hydrophobicity is usually expressed as the octanol-water partition coefficient (log Pow), where a high log Pow value corresponds to high hydrophobicity (Briggs *et al*., 1982; Ryan *et al*., 1988). Thus, substances with lipophilicity close to that to the respective plant root can be transferred from soil or water to the plant spontaneously by a diffusion-driven process. Organic compounds with log Pow ranging from 1 and 3 (moderately hydrophobic) are mostly taken up by plants (Briggs *et al*., 1982).

Furthermore, 24-Epibrassinolide is a moderately hydrophobic organic compound with a log Pow of 2.0 (please refer to MCA 2) and will be spontaneously transferred from soil or water to the plant by a diffusion-driven process and therefore not available for leaching to the different water bodies and systems. This was also confirmed by feeding experiments performed by Yokota *et al*. (1992) and Nishikawa *et al*. (1994). The authors showed that labelled Brassinosteroids was passively taken up by the roots and subsequently catabolised.

RMS Comments:
No valid information is available for the determination of the mobility of 24-Epibrassionolide in soil. The Notifier provides indications on the mobility of the active substance within the different parts of plants, and also bases its argumentation on the moderate hydrophobicity of the active substance to determine the mobility behaviour of the substance in soil. This information provides evidence of spontaneous transfer from soil or water to the plant by diffusion-driven process. RMS agrees with this argumentation but is only partially agreeing with the assumption that the active substance is therefore not available for leaching to the different water bodies or systems. Therefore, RMS recommends to use the default K_{fOC} of 0 L/kg as proposed by the Notifier but also to perform a second set of modelling using a default K_{fOC} value of 10000 L/kg.

CA 8.1.4.1 Column leaching studies

CA 8.1.4.1.1 Column leaching of the active substance

Study not considered necessary. Please see CA 8.1.4 above.

CA 8.1.4.1.2 Column leaching of metabolites, breakdown and reaction products

Study not considered necessary. Please see CA 8.1.4 above.

CA 8.1.4.2 Lysimeter studies

A lysimeter study is an optional higher tier study. In view of the ubiquitous natural occurrence, the rapid degradation and the low leaching potential of 24-Epibrasssinolide, a study is not considered necessary.

CA 8.1.4.3 Field leaching studies

Field leaching studies are optional higher tier studies. In view of the ubiquitous natural occurrence, the rapid degradation and the low leaching potential of 24-Epibrasssinolide, a study is not considered necessary.

CA 8.2 Fate and Behaviour in Water and Sediment

Potential effects of 24-Epibrassinolide on water treatment procedures

Brassinosteroids, including 24-Epibrassinolide are naturally occurring, plant growth promoting molecules, present in higher plants, lower plants, including algae, mosses, the "living fossil" Equisetum as well as some fungi (Takatsuto et al., 1990a, Table 6.2.1-1). Due to the constant formation and decomposition of plant root systems, the presence of seeds, pollen, and decomposing plant material and the release of Brassinosteroids from decomposing organic matter (e.g. Aremu et al., 2015) as well as the vast number of other Brassinosteroid producing organisms such as algae in the environment, Brassinosteroids – as other phytosterols – are naturally present in all environmental compartments including water-bodies and sediment (Hassett & Lee, 1977; Mudge et al., 1999). No impact on water treatment procedure is expected.

In addition to that, currently there is neither a guideline for testing the effect of water treatment on pesticides (or other chemicals) nor is there a risk assessment procedure. Since conditions of water treatment are extremely variable across Europe (different treatment methods and intensities used in different sequences on different types of raw waters) it is currently not possible to comprehensively assess the potential influence on water treatment procedures.

RMS Comments:
RMS agrees with the fact that phytosteroids are components naturally present in the environment and the water treatment procedures will probably not be affected by the active substance. On the other hand, the Notifier failed to provide evidence on the effect of water treatment plants on the active substance and more precisely the effect of chlorination or ozonification on the substance.

CA 8.2.1 Route and rate of degradation in aquatic systems (chemical and photochemical degradation)

CA 8.2.1.1 Hydrolytic degradation

Data point addressed:	CA 8.2.1.1/01
Author(s) (year):	Chen, S., Shi, L., Shan, Z., Hu, Q. (2005)
Title:	CHARACTERISTICS OF HYDROLYSIS AND DEGRADATION OF BRASSINOLIDE IN SOILS
Laboratory report / project Number (Doc. No.):	Not applicable (092-001)
Testing facility:	Not applicable
Published:	Yes (Rural Eco-Environment, 2005, 21 (1), 55-57)
Test guideline used:	Not indicated
Deviations:	None
GLP:	No

Executive Summary
Hydrolysis characteristics of Brassinolide at different pH values and temperatures and its degradation characteristics in four soils, including Northeast China black soil, sterilized Northeast China black soil, Jiangxi red soil, and Henan fluvo-aquic soil, were investigated. The aerobic degradation properties researched by the authors are described under CA 8.1.2.1.1.

Results show that hydrolysis of Brassinolide follow the first-order of kinetics. Its hydrolysis half-life at pH 5, 7 and 9 was 24.1, 19.6 and 16.4 days at 25 °C, and 20.9, 16.3 and 13.6 days at 50 °C, respectively.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material:

Brassinolide reference standard with a mass fraction greater than 99% was supplied by Yunda Group.

B. STUDY DESIGN

1. Experimental conditions

Brassinolide standard solution was prepared by accurately weighing 0.010 g Brassinolide reference standard and transferring it into a 100 mL volumetric flask. The Brassinolide was dissolved and diluted to the mark with methanol, thereby obtaining 100 mg/L standard solution. Working solution was prepared by diluting the standard solution with deionized water.

Brassinolide solution was respectively pipetted into buffer solutions with different pH values, and ultrasonicated for 0.5 h to enable complete dissolution of Brassinolide. The solutions obtained were transferred into 3 groups of 500mL reagent flasks (each group included 2 flasks). Reagent flasks in each group were placed into 25 °C and 50 °C thermostatic incubators respectively, and samples were collected periodically for determining the Brassinolide concentration in aqueous samples.

The vessels and buffer solutions for the hydrolysis experiment were autoclaved. Samples were protected against the influence of oxidation during the whole experiment process.

2. Extraction

50 mL aqueous sample was pipetted into a 125 mL separatory funnel, and extracted twice with 25 mL ethyl acetate. The aqueous phase was discarded. 5 mg phenylboronic acid was added to the organic phase, derivatised for 0.5 h in an 80 °C water bath, cooled and concentrated in a rotary evaporator. The concentrate was diluted to 5 mL with methanol for the HPLC determination of Brassinolide.

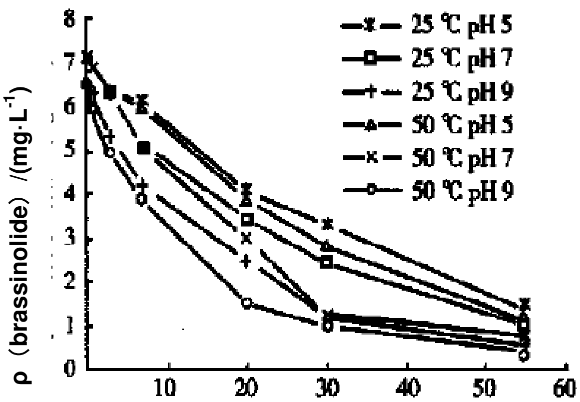
3. Analytical conditions

The chromatographic conditions were: Nucleosil 100 - 5 C8 stainless steel column (250 mm×4. 6 mm); mobile phase: V(acetonitrile) : V(water) = 75 : 25; flow rate: 1 mL/min; column temperature: 37 °C; detection wavelength: UV 222 nm; sensitivity: 0.2 AUFS. Brassinolide had a retention time (tR) of 5.6 min under the aforesaid chromatographic conditions.

The minimum detectable amount of Brassinolide was 2×10^{-9} g. For the determination of recovery rate, 0.5 - 10 mg Brassinolide per kg of soil was added. The recovery rate of Brassinolide was 84.3-93.2 %.

II. RESULTS AND DISCUSSION

The hydrolysis characteristics of substances are important indicators for the evaluation of their environmental stability. Hydrolysis reaction is a main pathway for dissipation of many substances in the environment. The hydrolysis pattern of Brassinolide was determined at different temperatures and pH values. The test results are shown in Figure 8.2.1.1-1 and Table 8.2.1.1-1.



Time (d)

Figure 8.2.1.1-1: Hydrolysis of Brassinolide in three buffer solutions with different pH values

Table 8.2.1.1-1: Kinetic parameters of the hydrolysis of Brassinolide in three buffer solutions different in temperature and pH value

Temperature, °C	pH value	Degradation rate, d^{-1}	Half-life, d
25	5	0. 030	24. 1
	7	0. 036	19.6
	9	0. 042	16.4
50	5	0. 034	20. 9
	7	0. 043	16.3
	9	0. 051	13.6

As shown in Figure 8.2.1.1-1 and Table 8.2.1.1-1, the hydrolysis of Brassinolide at different pH values and temperatures followed first-order kinetics: The half-life of Brassinolide at pH 5, 7, 9 was 24.1, 19.6, 16.4 days at 25 °C, and 20.9, 16.3 and 13.6 days at 50 °C, respectively.

The hydrolysis of Brassinolide was not influenced by the pH but also the temperature. Alkalinity and high temperature accelerated the hydrolysis of Brassinolide.

III. CONCLUSION

The hydrolysis of Brassinolide followed first-order kinetics. The degradation rate is mainly influenced by the pH, followed by the temperature. The highest degradation rate is measured at a pH of 9 and a temperature of 50 °C. At room temperature, Brassinolide has a hydrolysis half-life of less than 30 days at pH 5, 7 and 9 and according to the authors, can be considered as an easily hydrolysable substance. Therefore, it can be concluded that Brassinolide will quickly degrade in surface water bodies (around pH 7, temperature: 0 – 30 °C).

In addition to hydrolytic degradation, biological processes such as uptake of Brassinosteroids through aquatic plants and algae contribute to the overall fate of Brassinosteroids in water. Mekhalfi *et al.,* 2012 shows that 24-Epibrassinolide is readily taken up by algae and metabolized. Thus, due to the rapid uptake by algae, 24-Epibrassinolide is removed from water much faster than by hydrolysis alone.

RMS Comments:
The study provides information on the hydrolysis of 24-Epibrassinolide in three buffer solutions (pH 5, 7 and 9 at two temperatures 25°C and 50°C. The notifiers concluded that alkalinity and high temperature accelerated the hydrolysis of brassinolide, but the RMS could not identlify significant proof to support this statement.
RMS proposes to use the temperature normalised (for 20°C) value at pH 7 corresponding to 31.48 days for modelling of water-sediment systems.

CA 8.2.1.2 Direct photochemical degradation

Photolysis is not expected to contribute significantly to the degradation of 24-Epibrassinolide due to the low light absorbance of the active substance at a wavelength of 295 nm. Please refer to MCA 2.

CA 8.2.1.3 Indirect photochemical degradation

Photolysis is not expected to contribute significantly to the degradation of 24-Epibrassinolide due to the low light absorbance of the active substance at a wavelength of 295 nm. Please refer to MCA 2.

CA 8.2.2 Route and rate of biological degradation in aquatic systems

Brassinosteroids are ubiquitous in the environment, and naturally present in higher plants, lower plants, including algae and mosses and in certain fungi. Brassinosteroids are present in all plant organs such as pollens, anthers, seeds, leaves, stems, roots, flowers, grains and fruits with the highest concentrations found in pollen, seeds and fruits (Zhu *et al.*, 2013) and considered an obligatory plant constituent. The concentration of Brassinosteroids in plants is regulated by a complex system of feedback pathways and Brassinosteroids are constantly synthesised, metabolised, catabolised, activated and inactivated depending on the plant’s needs as well as environmental cues. The concentrations of Brassinosteroids are continuously fluctuating - spatially and temporally: in a single plant, different concentrations can be measured simultaneously in different plant organs, cell structures and cells as well as in the same location at different times.

Due to the constant formation and decomposition of plant root systems, the presence of seeds, pollen, and decomposing plant material and the release of Brassinosteroids from decomposing organic matter (e.g. Aremu *et al.*, 2015) as well as the vast number of other Brassinosteroid producing organisms such as algae in the environment, Brassinosteroids are expected to be naturally present in all environmental compartments including soil and water-bodies as well as sediment (Aremu *et al.*, 2015, Hassett and Lee, 1977; Mudge *et al.* 1999). No different degradation pathway of the natural-identical synthesized molecule, 24-Epibrassinolide, to the natural occurring 24-Epibrassinolide, are expected.

Brassinosteroids are metabolized by certain microorganisms. 24-Epicastasterone and 24-Epibrassinolide are transformed by the fungus *Cunninghamella echinulata* to give the corresponding 12β-hydroxylated compounds (e.g. 12β-hydroxy-24-Epibrassinolide) (Voigt *et al.*, 1993). The degradation of Brassinosteroids by the fungus *Cochliobolus lunatus* and the *Mycobacterium vaccae* was also reported by Voigt *et al*. (1993) and Vorbrodt *et al*. (1991).

The growth promoting and increased shelf life effects shown by the application of Brassinosteroids to the mushroom cultures of *Psilocybe cubensis* (Gartz, 1990), *Agaricus bisporus* (Ding *et al.*, 2016) and *Pleurotus ostratus* (Khripach *et al.*, 2003) show that Brassinosteroids are absorbed and metabolized by mushroom cultures.

Brassinosteroids also have a strengthening and activating effect on Cyanobacteria, *Spirulina platensis* (Saygideger and Deniz, 2008) and the diatom *Asterionella formosa* when applied (Mekhalfi *et al.*, 2012), showing that these microorganisms are able to take up and metabolise Brassinosteroids. It is therefore proposed that 24-Epibrassinolide is removed from water much faster than by hydrolysis alone (see CA 8.2.1.1).

In addition to that, bioaccumulation is not expected as Brassinosteroids are readily absorbed and metabolised by higher and lower plants (e.g. Nishikawa *et al*, 1994), diatoms (e.g. Mekhalfi *et al.,* 2012), green algae (e.g. Bajguz, 2011), fungi (e.g. Voigt *et al*., 1993), mycobacteria (e.g. Vorbrodt *et al*., 1991), and cyanobacteria (e.g. Saygideger and Deniz, 2008). As Brassinosteroids are phylogenetically ancient phytohormones, it can be expected that each organism has developed its own co-evolutionary mechanism to metabolise these phytohormones.

RMS Comments:
The argumentation provided by the Notifier is mainly based on the presence of phytosteroids in soils, plants and fungi. The effects on cyanobacteria and on diatom are the only evidence of the influence of brassinosteroids in the aquatic compartment. Furthermore, absorption and metabolism of brassinosteroids were investigated in higher and lower plants, diatoms, green algae, fungi, mycobacteria, and cyanobacteria. Unfortunately, no rate of degradation in water-sediment systems could be determined from the open literature and the proposition of the Notifier that 24-Epibrassinolide is removed from water much faster than by hydrolysis alone could not be verified.
RMS proposes to use the degradation value of hydrolysis at pH 7 and normalized to 20°C. This value appears to describe the expected behaviour of 24-Epibrassinolide in water. RMS estimates the use of a default value of 1000 days would not be representative for 24-Epibrassinolide.

CA 8.2.2.1 "Ready biodegradability"

Due to the constant formation and decomposition of plant root systems, the presence of seeds, pollen, and decomposing plant material and the release of Brassinosteroids from decomposing organic matter (e.g. Aremu *et al*., 2015) as well as the vast number of other Brassinosteroid producing organisms such as algae in the environment, Brassinosteroids are expected to be naturally present in all environmental compartments including soil and water-bodies including sediment (Aremu *et al*., 2015, Hassett and Lee, 1977; Mudge *et al*., 1999).

A substance can be considered "readily biodegreable" according to REGULATION (EC) No 1272/2008 "if other convincing scientific evidence is available to demonstrate that the substance can be degraded (biotically and/or abiotically) in the aquatic environment to a level > 70 % within a 28-day period."

As was shown in the hydrolytic degradation study, half-life of Brassinolide was 24.1 days at pH 5 at 25 °C. Together with biological degradation through readily uptake of 24-Epibrassinolide by algae (Mekhalfi *et al*., 2012) and plants, it can be concluded that the degradability in water will be at a level >70 % within a 28-day period. It is therefore concluded that 24-Epibrassinolide is readily biodegradable and a study is not considered necessary.

RMS Comments:
RMS can not fully agree on the fact that enough data is available to prove that >70 % of 24-epibrassinolide would be metabolised within a 28-day period based on the half-life value for 24-Epibrassinolide of 24.1 days at pH 5 at 25 °C. RMS would propose to classify 24-epibrassinolide as non-readily biodegradable.

CA 8.2.2.2 Aerobic mineralisation in surface water

A study on the aerobic mineralisation in surface water is not considered necessary; please refer to CA 8.2.2 above.

CA 8.2.2.3 Water/sediment studies

A water/sediment study is not considered necessary; please refer to CA 8.2.2 above.

CA 8.2.2.4 Irradiated water/sediment study

As impact of irradiation on degradation in water/sediment systems is expected to be limited, an irradiated water/sediment study is not deemed necessary.

CA 8.2.3 Degradation in the Saturated Zone

An aged sorption study is an optional higher tier study. In view of the natural ubiquitous distribution of Brassinosteroids and the low leaching potential of 24-epibrasssinolide, respective data is not considered necessary.

CA 8.3 Fate and Behaviour in Air

No special experimental data are available on the active substance 24-Epibrassinolide or its products in air. However, 24-Epibrassinolide is naturally occurring and has a low volatility (vapour pressure: 1.90 x 10^{-15} Pa; calculated Henry's law constant 2.40 x 10^{-13} $Pa \cdot m^3/mol$) and hence it is not considered to pose any significant concern in air.

CA 8.3.1 Route and rate of degradation in air

A study on the route of degradation in air is not considered necessary; please refer to CA 8.3 above.

CA 8.3.2 Transport via air

The vapour pressure of 1.90 x 10^{-15} Pa of 24-Epibrassinolide is significantly below the trigger for volatilisation of 1×10^{-5} Pa for plants and 1×10^{-4} Pa for soil. Volatilisation from plants and soil are not likely to occur.

RMS Comments:
RMS agrees with the conclusions proposed by the Notifier on the behaviour of 24-Epibrassinolide in air.

CA 8.3.3 Local and global effects

Neither local nor global effects as described in the EU Regulation No. 283/2013 are expected.

CA 8.4 Definition of the Residue

Brassinosteroids, including 24-Epibrassinolide are naturally occurring, plant growth promoting molecules, found at low concentrations throughout the plant kingdom. They are present in higher plants, lower plants, including algae and mosses and in some fungi (Table 7-2).

Due to the constant formation and decomposition of plant root systems, the presence of seeds, pollen, and decomposing plant material and the release of Brassinosteroids from decomposing organic matter (e.g. Aremu *et al*., 2015) as well as the vast number of other Brassinosteroid producing organisms such as algae in the environment, as for other phytosterols, Brassinosteroids and their breakdown products are naturally present in all environmental compartments including soil and water-bodies as well as sediment (Aremu *et al*., 2015, Hassett and Lee, 1977; Mudge *et al*., 1999).

However, it is impossible to distinguish between a possible residue resulting from the use of the natural-identical active substance 24-Epibrassinolide and the similar natural substance ubiquitously present in the environment.

Moreover, 24-Epibrassinolide is expected to be readily metabolised into elements naturally present in the environment.

The relevance of a residue definition in environmental compartments is therefore not considered necessary, since the origin of the active substance measured in the environmental samples cannot be established with certainty (i.e. natural source or from treatment). Therefore, there is no need to propose a residue definition for 24-Epibrassinolide in any environmental compartment.

CA 8.4.1 Definition of the residue for risk assessment

A residue definition is not considered necessary; please refer to CA 8.4 above.

CA 8.4.2 Definition of the residue for monitoring

A residue definition is not considered necessary; please refer to CA 8.4 above.

CA 8.5 Monitoring Data

No monitoring data in the different environmental compartments of the active substance 24-Epibrasssinolide and its metabolites have been reported. However, Brassinosteroids, including 24-Epibrassinolide are naturally occurring molecules found at low concentrations throughout the plant kingdom and some fungi, which have been the subject of decade-long scientific studies e.g. regarding the occurrence in the environment (e.g. Hassett and Lee, 1977). Please refer to Table 7-2 above for a summary of the measured concentrations.

Publications to support evaluation

Report:	CA 8/01, Takatsuto, S., Abe, H., Gamoah, K., 1990 a
Title:	EVIDENCE FOR BRASSINOSTEROIDS IN STROBILUS OF EQUISETUM ARVENSE L.
Laboratory report / project number (Doc. No.)	Not applicable (092-059)
Guidelines:	Not indicated
Published:	Yes (Agricultural and Biological Chemistry, 1990, 54 (4), 1057-1059)
GLP:	No

Report:	CA 8/02, Zhu, J.-Y., Sae-Seaw, J., Wang, Z.-Y., 2013
Title:	BRASSINOSTEROID SIGNALLING
Laboratory report / project number (Doc. No.)	Not applicable (092-165)
Guidelines:	Not indicated
Published:	Yes (Development, 2013, 140(8), 1615-1620; doi: 10.1242/dev.060590)
GLP:	No

Report:	CA 8/03, Saini, S., Sharma, I., Pati, P.K., 2015
Title:	VERSATILE ROLES OF BRASSINOSTEROID IN PLANTS IN THE CONTEXT OF ITS HOMOEOSTASIS, SIGNALING AND CROSSTALKS
Laboratory report / project number (Doc. No.)	Not applicable (092-182)
Guidelines:	Not indicated
Published:	Yes (Frontiers in Plant Science, 2015, 6, 950; doi: 10.3389/fpls.2015.00950)
GLP:	No

Report:	CA 8/04, Symons, G.M., Ross, J.J., Jager, C.E., Reid, J.B., 2008
Title:	BRASSINOSTEROID TRANSPORT
Laboratory report / project number (Doc. No.)	Not applicable (092-094)
Guidelines:	Not indicated
Published:	Yes (Journal of Experimental Botany, 2008, 59 (1), 17-24; doi:10.1093/jxb/erm098)
GLP:	No

Report:	CA 8/05, Kutschera, U., Wang, Z.-Y., 2012
Title:	BRASSINOSTEROID ACTION IN FLOWERING PLANTS: A DARWINIAN PERSPECTIVE
Laboratory report / project number (Doc. No.)	Not applicable (092-036)
Guidelines:	Not indicated
Published:	Yes (Journal of Experimental Botany, 2012, 63 (10), 3511-3522; doi:10.1093/jxb/ers065)
GLP:	No

Report:	CA 8/06, Thompson, M.J., Mandava, N., Flippen-Anderson, J.L., Worley, J.F., Dutky, S.R., Robbins, W.E., Lusby, W., 1979
Title:	SYNTHESIS OF BRASSINO STEROIDS: NEW PLANT-GROWTH-PROMOTING STEROIDS
Laboratory report / project number (Doc. No.)	Not applicable (092-063)
Guidelines:	Not indicated
Published:	Yes (The Journal of Organic Chemistry, 1979, 44 (26), 5002-5004)
GLP:	No

Report:	CA 8/07, Ikekawa, N., Nishiyama, F., Fujimoto, Y., 1988
Title:	IDENTIFICATION OF 24-EPIBRASSINOLIDE IN BEE POLLEN OF THE BROAD BEAN, VICIA FABA L.
Laboratory report / project number (Doc. No.)	Not applicable (092-027)
Guidelines:	Not indicated
Published:	Yes (Chemical and Pharmaceutical Bulletin, 1988, 36 (1), 405-407)
GLP:	No

Report:	CA 8/08, Bajguz, A., 2011
Title:	SUPPRESSION OF CHLORELLA VULGARIS GROWTH BY CADMIUM, LEAD, AND COPPER STRESS AND ITS RESTORATION BY ENDOGENOUS BRASSINOLIDE
Laboratory report / project number (Doc. No.)	Not applicable (092-103)
Guidelines:	Not indicated
Published:	Yes (Archives of Environmental Contamination and Toxicology, 2011, 60, 406-416; DOI 10.1007/s00244-010-9551-0)
GLP:	No

Report:	CA 8/09, Khripach, V., Zhabinskii, V., De Groot, A., 2000
Title:	TWENTY YEARS OF BRASSINOSTEROIDS: STEROIDAL PLANT HORMONES WARRANT BETTER CROPS FOR THE XXI CENTURY
Laboratory report / project number (Doc. No.)	Not applicable (092-029)
Guidelines:	Not indicated
Published:	Yes (Annals of Botany, 2000, 86, 441-447; doi:10.1006/anbo.2000.1227)
GLP:	No

Report:	CA 8/10, Ikekawa, N., Zhao, Y.-J., 1991
Title:	APPLICATION OF 24-EPIBRASSINOLIDE IN AGRICULTURE
Laboratory report / project number (Doc. No.)	Not applicable (092-026)
Guidelines:	Not indicated
Published:	Yes (ACS Symposium series, 1991, 474, Chapter 24, 280-291)
GLP:	No

Report:	CA 8/11, Bajguz, A., Tretyn, A., 2003
Title:	THE CHEMICAL STRUCTURES AND OCCURRENCE OF BRASSINOSTEROIDS IN PLANTS
Laboratory report / project number (Doc. No.)	Not applicable (092-145)
Guidelines:	Not indicated
Published:	Yes (Brassinosteroids. Chapter 1, 2003, 1-44)
GLP:	No

Report:	CA 8/12, Hayat, s., Ahmad, A., 2011
Title:	BRASSINOSTEROIDS: A CLASS OF PLANT HORMONE
Laboratory report / project number (Doc. No.)	Not applicable (092-146)
Guidelines:	Not indicated
Published:	Yes (Springer Verlag, 2011, 1-477, DOI 10.1007/978-94-007-0189-2; ISBN: 978-94-007-0188-5)
GLP:	No

Report:	CA 8/13, Abe, H., Nakamura, K., Morishita, T., Uchiyama, M., Takatsuto, S., Ikekawa, N., 1984 b
Title:	ENDOGENOUS BRASSINOSTEROIDS OF THE RICE PLANT: CASTASTERONE AND DOLICHOSTERONE
Laboratory report / project number (Doc. No.)	Not applicable (092-004)
Guidelines:	Not indicated
Published:	Yes (Agricultural and Biological Chemistry, 1984, 48 (4), 1103-1104)
GLP:	No

Report:	CA 8/14, Abe, H., Takatsuto, S., Nakayama, M., Yokota, T., 1995 a
Title:	28-HOMOTYPHASTEROL, A NEW NATURAL BRASSINOSTEROID FROM RICE (ORYZA SATIVA L.) BRAN
Laboratory report / project number (Doc. No.)	Not applicable (092-006)
Guidelines:	Not indicated
Published:	Yes (Bioscience, Biotechnology and Biochemistry, 1995, 59 (2), 176-178)
GLP:	No

Report:	CA 8/15, Park, K.-H., Park, J.-D., Hyun, K.-H., Nakayama, M., Yokota, T., 1994 b
Title:	BRASSINOSTEROIDS AND MONOGLYCERIDES IN IMMATURE SEEDS OF CASSIA TORA AS THE ACTIVE PRINCIPLES IN THE RICE LAMINA INCLINATION BIOASSAY
Laboratory report / project number (Doc. No.)	Not applicable (092-046)
Guidelines:	Not indicated
Published:	Yes (Bioscience, Biotechnology and Biochemistry, 1994, 58 (7), 1343-1344)
GLP:	No

Report:	CA 8/16, Khripach, V.A., Litvinovskaya, R.P., Kurtikova, A.L., Drach, S.V., Pryadko, A.G., Mirantsova, T.V., Baranovskiy, A.V., 2013
Title:	ENZYME IMMUNOASSAY OF THE CONTENT OF ENDOGENOUS BRASSINOSTEROIDS IN PHYTOGENIC FOOD PRODUCTS
Laboratory report / project number (Doc. No.)	Not applicable (092-030)
Guidelines:	Not indicated
Published:	Yes (National Academy of Sciences of Belarus, 2013, 57 (2), 63-69)
GLP:	No

Report:	CA 8/17, Yokota, T., Nakayama, M., Wakisaka, T., Schmidt, J., Adam, G., 1994
Title:	3-DEHYDROTEASTERONE, A 3,6-DIKETOBRASSINOSTEROID AS A POSSIBLE BIOSYNTHETIC INTERMEDIATE OF BRASSINOLIDE FROM WHEAT GRAIN
Laboratory report / project number (Doc. No.)	Not applicable (092-078)
Guidelines:	Not indicated
Published:	Yes (Bioscience, Biotechnology and Biochemistry, 1994, 58 (6), 1183-1185)
GLP:	No

Report:	CA 8/18, Suzuki, Y., Yamaguchi, I., Yokota, T., Takahasi, N., 1986
Title:	IDENTIFICATION OF CASTASTERONE, TYPHASTEROL AND TEASTERONE FROM THE POLLEN OF ZEA MAYS

Laboratory report / project number (Doc. No.)	Not applicable (092-053)
Guidelines:	Not indicated
Published:	Yes (Agricultural and Biological Chemistry, 1986, 50 (12), 3133-3138)
GLP:	No

Report:	CA 8/19, Kim, S.-K., Chang, S.C., Lee, E.J., Chung, W.-S., Kim, Y.-S., Hwang, S., Lee, J.S., 2000 a
Title:	INVOLVEMENT OF BRASSINOSTEROIDS IN THE GRAVITROPIC RESPONSE OF PRIMARY ROOT OF MAIZE
Laboratory report / project number (Doc. No.)	Not applicable (092-034)
Guidelines:	Not indicated
Published:	Yes (Plant Physiology, 2000, 123, 997-1004)
GLP:	No

Report:	CA 8/20, Yasuta, E., Terahata, T., Nakayama, M., Abe, H., Takatsuto, S., Yokota, T., 1995
Title:	FREE AND CONJUGATED BRASSINOSTEROIDS IN THE POLLEN AND ANTHERS OF ERYTHRONIUM JAPONICUM DECNE
Laboratory report / project number (Doc. No.)	Not applicable (092-067)
Guidelines:	Not indicated
Published:	Yes (Bioscience, Biotechnology and Biochemistry, 1995, 59 (11), 2156-2158)
GLP:	No

Report:	CA 8/21, Suzuki, H., Fujioka, S., Yokota, T., Murofushi, N., Sakurai, A., 1994 b
Title:	IDENTIFICATION OF BRASSINOLIDE, CASTASTERONE, TYPHASTEROL AND TEASTERONE FROM THE POLLEN OF LILIUM ELEGANS
Laboratory report / project number (Doc. No.)	Not applicable (092-054)
Guidelines:	Not indicated
Published:	Yes (Bioscience, Biotechnology and Biochemistry, 1994, 58 (11), 2075-2076)
GLP:	No

Report:	CA 8/22, Abe, H., Honjo, C., Kyokawa, Y., Asakawa, S., Natsume, M., Narushima, M., 1994
Title:	3-OXOTEASTERONE AND THE EPIMERIZATION OF TEASTERONE: IDENTIFICATION IN LILY ANTHERS AND DISTYLIUM RACEMOSUM LEAVES AND ITS BIOTRANSFORMATION INTO TYPHASTEROL
Laboratory report / project number (Doc. No.)	Not applicable (092-005)
Guidelines:	Not indicated
Published:	Yes (Bioscience, Biotechnology and Biochemistry, 1994, 58 (5), 986-989)
GLP:	No

Report:	CA 8/23, Asakawa, S., Abe. H., Kyokawa, Y., Nakamura, S., Natsume, M., 1994
Title:	TEASTERONE 3-MYRISTATE: A NEW TYPE OF BRASSINOSTEROID DERIVATIVE IN LILIUM LONGIFLORUM ANTHERS
Laboratory report / project number (Doc. No.)	Not applicable (092-009)
Guidelines:	Not indicated
Published:	Yes (Bioscience, Biotechnology and Biochemistry, 1994, 58 (1), 219-220)
GLP:	No

Report:	CA 8/24, Asakawa, S., Abe, H., Nishikawa, N., Natsume, M., Koshioka, M., 1996
Title:	PURIFICATION AND IDENTIFICATION OF NEW ACYL-CONJUGATED TEASTERONES IN LILY POLLEN
Laboratory report / project number (Doc. No.)	Not applicable (092-010)
Guidelines:	Not indicated
Published:	Yes (Bioscience, Biotechnology and Biochemistry, 1996, 60 (9), 1416-1420)
GLP:	No

Report:	CA 8/25, Soene, K., Kyokawa, Y., Natsume, M., Abe, H., 2000
Title:	TEASTERONE-3-O-ß-D-GLUCOPYRANOSIDE, A NEW CONJUGATED BRASSINOSTEROID METABOLITE FROM LILY CELL SUSPENSION CULTURES AND ITS IDENTIFICATION IN LILY ANTHERS
Laboratory report / project number (Doc. No.)	Not applicable (092-050)
Guidelines:	Not indicated
Published:	Yes (Bioscience, Biotechnology and Biochemistry, 2000, 64 (4), 702-709)
GLP:	No

Report:	CA 8/26, Plattner, R.D., Taylor, S.L., Grove, M.D., 1986
Title:	DETECTION OF BRASSINOLIDE AND CASTASTERONE IN ALNUS GLUTINOSA (EUROPEAN ALDER) POLLEN BY MASS SPECTROMETRY/MASS SPECTROMETRY
Laboratory report / project number (Doc. No.)	Not applicable (092-047)
Guidelines:	Not indicated
Published:	Yes (Journal of Natural Products, 1986, 49 (3), 540-545)
GLP:	No

Report:	CA 8/27, Takatsuto, S., Abe, H., Yokota, T., Shimada, K., Gamoh, K., 1996 b
Title:	IDENTIFICATION OF CASTASTERONE AND TEASTERONE IN SEEDS OF CANNABIS SATIVA L.
Laboratory report / project number	Not applicable (092-062)

(Doc. No.)	
Guidelines:	Not indicated
Published:	Yes (Japan Oil Chemists' Society, 1996, 45 (9), 871-873)
GLP:	No

Report:	CA 8/28, Schmidt, J., Boehme, F., Adam, G., 1996
Title:	24-EPIBRASSINOLIDE FROM GYPSOPHILA PERFOLIATA
Laboratory report / project number (Doc. No.)	Not applicable (092-049)
Guidelines:	Not indicated
Published:	Yes (Zeitschrift für Naturforschung, 1996, 51 c, 897-899)
GLP:	No

Report:	CA 8/29, Yokota, T., Arima, M., Takahashi, N., 1982 a
Title:	CASTASTERONE, A NEW PHYTOSTEROL WITH PLANT-HORMONE POTENCY, FROM CHESTNUT INSECT GALL
Laboratory report / project number (Doc. No.)	Not applicable (092-072)
Guidelines:	
Published:	Yes (Tetrahedron letters, 1982, 23 (12), 1275-1278)
Abstract:	A sterol which has strong potency to inclinate rice lamina was isolated from the insect gall of the chestnut tree. The structure was determined to be (22R, 23R, 24S)-2?, 3?, 22, 23-tetrahydroxy-24-methyl-5?-cholestan-6-one (1).
GLP:	No

Report:	CA 8/30, Ikeda, M., Takatsuto, S., Sassa, T., Ikekawa, N., Nukina, M., 1983
Title:	IDENTIFICATION OF BRASSINOLIDE AND ITS ANALOGUES IN CHESTNUT GALL TISSUE
Laboratory report / project number (Doc. No.)	Not applicable (092-024)
Guidelines:	Not indicated
Published:	Yes (Agricultural and Biological Chemistry, 1983, 47 (3), 655-657)
GLP:	No

Report:	CA 8/31, Ikekawa, N., Takatsuto, S., 1984
Title:	MICROANALYSIS OF BRASSIOSTEROIDS IN PLANTS BY GAS CHROMATOGRAPHY/MASS SPECTROMETRY
Laboratory report / project number (Doc. No.)	Not applicable (092-025)
Guidelines:	Not indicated
Published:	Yes (Mass Spectroscopy, 1984, 32 (1), 55-70)
GLP:	No

Report:	CA 8/32, Asakawa, S., Abe. H., Kyokawa, Y., Nakamura, S., Natsume, M., 1994
Title:	TEASTERONE 3-MYRISTATE: A NEW TYPE OF BRASSINOSTEROID DERIVATIVE IN LILIUM LONGIFLORUM ANTHERS
Laboratory report / project number (Doc. No.)	Not applicable (092-009)
Guidelines:	Not indicated
Published:	Yes (Bioscience, Biotechnology and Biochemistry, 1994, 58 (1), 219-220)
GLP:	No

Report:	CA 8/33, Arima, M., Yokota, T., Takahashi, N., 1984
Title:	IDENTIFICATION AND QUANTIFICATION OF BRASSINOLIDE-RELATED STEROIDS IN THE INSECT GALL AND HEALTHY TISSUES OF THE CHESTNUT PLANT
Laboratory report / project number (Doc. No.)	Not applicable (092-008)
Guidelines:	Not indicated
Published:	Yes (Phytochemistry, 1984, 23 (8), 1587-1591)
GLP:	No

Report:	CA 8/34, Takatsuto, S., Omote, K., Gamoh, K., Ishibashi, M., 1990 b
Title:	IDENTIFICATION OF BRASSINOLIDE AND CASTASTERONE IN BUCKWHEAT (FAGOPYRUM ESCULENTUM MOENCH) POLLEN
Laboratory report / project number (Doc. No.)	Not applicable (092-060)
Guidelines:	Not indicated
Published:	Yes (Agricultural and Biological Chemistry, 1990, 54 (3), 757-762)
GLP:	No

Report:	CA 8/35, Sondhi, N., Bhardwaj, R., Kaur, S., Chandel, M., Kumar, N., Singh, B., 2010
Title:	INHIBITION OF H2O2-INDUCED DNA DAMAGE IN SINGLE CELL GEL ELECTROPHORESIS ASSAY (COMET ASSAY) BY CASTASTERONE ISOLATED FROM LEAVES OF CENTELLA ASIATICA
Laboratory report / project number (Doc. No.)	Not applicable (092-155)
Guidelines:	Not indicated
Published:	Yes (Health, 2010, 2 (6), 595-602; doi:10.4236/health.2010.26088)
GLP:	No

Report:	CA 8/36, Swaczynova, J., Novak, O., Hauserova, E., Fuksova, K., Sisa, M., Kohout, L., Strnad, M., 2007
Title:	NEW TECHNIQUES FOR THE ESTIMATION OF NATURALLY OCCURRING BRASSINOSTEROIDS
Laboratory report / project number (Doc. No.)	Not applicable (092-057)
Guidelines:	Not indicated
Published:	Yes (Journal of Plant Growth Regulation, 2007, 26, 1-14; DOI: 10.1007/s00344-006-0045-2)
GLP:	No

Report:	CA 8/37, Fujioka, S., Choi, Y.-H., Takatsuto, S., Yokota, T., Li, J., Chory, J., Sakurai, A., 1996
Title:	IDENTIFICATION OF CASTASTERONE, 6-DEOXOCASTASTERONE, TYPHASTEROL AND 6-DEOXOTYPHASTEROL FROM THE SHOOTS OF ARABIDOPSIS THALIANA
Laboratory report / project number (Doc. No.)	Not applicable (092-018)
Guidelines:	Not indicated
Published:	Yes (Plant & Cell Physiology, 1996, 37 (8), 1201-1203)
GLP:	No

Report:	CA 8/38, Fujioka, S., Li, J., Choi, Y.-H., Seto, H., Takatsuto, S., Noguchi, T., Watanabe, T., Kuriyama, H., Yokota, T., Chory, J., Sakurai, A., 1997
Title:	THE ARABIDOPSIS DEETIOLATED2 MUTATNT IS BLOCKED EARLY IN BRASSINOSTEROID BIOSYNTHESIS
Laboratory report / project number (Doc. No.)	Not applicable (092-019)
Guidelines:	Not indicated
Published:	Yes (The Plant Cell, 1997, 9, 1951-1962)
GLP:	No

Report:	CA 8/39, Nomura, T., Sato, T., Bishop, G.J., Kamiya, Y., Takatsuto, S., Yokota, T., 2001
Title:	ACCUMULATION OF 6-DEOXOCATHASTERONE AND 6-DEOXOCASTASTERONE IN ARABIDOPSIS, PEA AND TOMATO IS SUGGESTIVE OF COMMON RATE-LIMITING STEPS IN BRASSINOSTEROID BIOSYNTHESIS
Laboratory report / project number (Doc. No.)	Not applicable (092-040)
Guidelines:	Not indicated
Published:	Yes (Phytochemistry, 2001, 57, 171-178)
GLP:	No

Report:	CA 8/40, Bancos, S., Nomura, T., Sato, T., Molnar, G., Bishop, G.J., Koncz, C., Yokota, T., Nagy, F., Szekeres, M., 2002
Title:	REGULATION OF TRANSCRIPT LEVELS OF THE ARABIDOPSIS CYTOCHROME P450 GENES INVOLVED IN BRASSINOSTEROID BIOSYNTHESIS
Laboratory report / project number (Doc. No.)	Not applicable (092-161)
Guidelines:	Not indicated
Published:	Yes (Plant Physiology, 2002, 130, 504-513; DOI: 10.1104/pp.005439)
GLP:	No

Report:	CA 8/41, Schmidt, J., Altmann, T., Adam, G., 1997
Title:	BRASSINOSTEROIDS FROM SEEDS OF ARABIDOPSIS THALIANA
Laboratory report / project number (Doc. No.)	Not applicable (092-048)
Guidelines:	Not indicated
Published:	Yes (Phytochemistry, 1997, 45 (7), 1325-1327)
GLP:	No

Report:	CA 8/42, Choe, S., Fujioka, S., Noguchi, T., Takatsuto, S., Yoshida, S., Feldmann, K.A., 2001
Title:	OVEREXPRESSION OF DWARF4 IN THE BRASSINOSTEROID BIOSYNTHETIC PATHWAY RESULTS IN INCREASED VEGETATIVE GROWTH AND SEE YIELD IN ARABIDOPSIS
Laboratory report / project number (Doc. No.)	Not applicable (092-015)
Guidelines:	Not indicated
Published:	Yes (The Plant Journal, 2001, 26 (6), 573-582)
GLP:	No

Report:	CA 8/43, Fujioka, S., Takatsuto, S., Yoshida, S., 2002
Title:	AN EARLY C-22 OXIDATION BRANCH IN THE BRASSINOSTEROID BIOSYNTHETIC PATHWAY
Laboratory report / project number (Doc. No.)	Not applicable (092-020)
Guidelines:	Not indicated
Published:	Yes (Plant Physiology, 2002, 130 (2), 930-939; doi/10.1104/pp.008722)
GLP:	No

Report:	CA 8/44, Shimada, Y., Goda, H., Nakamura, A., Takatsuto, S., Fujioka, S., Yoshida, S., 2003
Title:	ORGAN-SPECIFIC EXPRESSION OF BRASSINOSTEROID-BIOSYNTHETIC GENES AND DISTRIBUTION OF ENDOGENOUS BRASSINOSTEROIDS IN ARABIDOPSIS
Laboratory report / project number (Doc. No.)	Not applicable (092-162)
Guidelines:	Not indicated
Published:	Yes (Plant Physiology, 2003, 131, 287-297; DOI: 10.1104/pp.013029)
GLP:	No

Report:	CA 8/45, Abe, H., Morishita, T., Uchiyama, M., Marumo, S., Munakata, K., Takatsuto, S., Ikekawa, N., 1982
Title:	IDENTIFICATION OF BRASSINOLIDE-LIKE SUBSTANCES IN CHINESE CABBAGE
Laboratory report / project number (Doc. No.)	Not applicable (092-002)
Guidelines:	Not indicated
Published:	Yes (Agricultural and Biological Chemistry, 1982, 46 (10), 2609-2611)
GLP:	No

Report:	CA 8/46, Ikekawa, N., Takatsuto, S., 1984
Title:	MICROANALYSIS OF BRASSIOSTEROIDS IN PLANTS BY GAS CHROMATOGRAPHY/MASS SPECTROMETRY

Laboratory report / project number (Doc. No.)	Not applicable (092-025)
Guidelines:	Not indicated
Published:	Yes (Mass Spectroscopy, 1984, 32 (1), 55-70)
GLP:	No

Report:	CA 8/47, Kanwar, M.K., Bhardwaj, R., Chowdhary, S.P., Arora, P., Sharma, P., Kumar, S., 2013
Title:	ISOLATION AND CHARACTERIZATION OF 24-EPIBRASSINOLIDE FROM BRASSICA JUNCEA L. AND ITS EFFECTS ON GROWTH, NI ION UPTAKE, ANTIOXIDANT DEFENSE OF BRASSICA PLANTS AND IN VITRO CYTOTOXICTY
Laboratory report / project number (Doc. No.)	Not applicable (092-118)
Guidelines:	Not indicated
Published:	Yes (Acta Physiologiae Plantarum, 2013, 35, 1351-1362; DOI 10.1007/s11738-012-1175-8)
GLP:	No

Report:	CA 8/48, Grove, M.D., Spencer, G.F., Rohwedder, W.K., Mandava, N., Worley, J.F., Warthen, J.D., Steffens, G.L., Flippen-Anderson, J.L., Cook, J.C., 1979
Title:	BRASSINOLIDE, A PLANT GROWTH-PROMOTING STEROID ISOLATED FROM BRASSICA NAPUS POLLEN
Laboratory report / project number (Doc. No.)	Not applicable (092-022)
Guidelines:	Not indicated
Published:	Yes (Nature, 1979, 281, 216-217)
GLP:	No

Report:	CA 8/49, Pan, J., Hu, Y., Liang, T., Li, G., 2012
Title:	PREPARATION OF SOLID-PHASE MICROEXTRACTION FIBERS BY IN-MOLD COATING STRATEGY FOR DERIVATIZATION ANALYSIS OF 24-EPIBRASSINOLIDE IN POLLEN SAMPLES
Laboratory report / project number (Doc. No.)	Not applicable (092-041)
Guidelines:	Not indicated
Published:	Yes (Journal oh Chromatography A, 2012, 1262, 49-55; doi: 10.1016/j.chroma.2012.09.008)
GLP:	No

Report:	CA 8/50, Baba, J., Yokota, T., Takahashi, N., 1983
Title:	BRASSINOLIDE-RELATED NEW BIOACTIVE STEROIDS FROM DOLICHOS LABLAB SEED
Laboratory report / project number	Not applicable (092-011)

(Doc. No.)	
Guidelines:	Not indicated
Published:	Yes (Agricultural and Biological Chemistry, 1983, 47 (3), 659-661)
GLP:	No

Report:	CA 8/51, Yokota, T., Baba, J., Takahashi, N., 1983 b
Title:	BRASSINOLIDE-RELATED BIOACTIVE STEROLS IN DOLICHOS LABLAB: BRASSINOLIDE, CASTASTERONE AND A NEW ANALOG HOMODOLICHOLIDE
Laboratory report / project number (Doc. No.)	Not applicable (092-073)
Guidelines:	Not indicated
Published:	Yes (Agricultural and Biological Chemistry, 1983, 47 (6), 1409-1411)
GLP:	No

Report:	CA 8/52, Yokota, T., Baba, J., Koba, S., Takahashi, N., 1984
Title:	PURIFICATION AND SEPARATION OF EIGHT STEROIDAL PLANT-GROWTH REGULATORS FROM DOLICHOS LABLAB SEED
Laboratory report / project number (Doc. No.)	Not applicable (092-075)
Guidelines:	Not indicated
Published:	Yes (Agricultural and Biological Chemistry, 1984, 48 (10), 2529-2534)
GLP:	No

Report:	CA 8/53, Abe, H., Takatsuto, S., Okuda, R., Yokota, T., 1995 b
Title:	IDENTIFICATION OF CASTASTERONE, 6-DEOXOCASTASTERONE, AND TYPHASTEROL IN THE POLLEN OF ROBINIA PSEUDO-ACACIA L.
Laboratory report / project number (Doc. No.)	Not applicable (092-007)
Guidelines:	Not indicated
Published:	Yes (Bioscience, Biotechnology and Biochemistry, 1995, 59 (2), 309-310)
GLP:	No

Report:	CA 8/54, Park, K.-H., Yokota, T., Sakurai, A., Takahashi, N., 1987
Title:	OCCURRENCE OF CASTASTERONE, BRASSINOLIDE AND METHYL 4-CHLOROINDOLE-3-ACETATE IN IMMATURE VICIA FABA SEEDS
Laboratory report / project number (Doc. No.)	Not applicable (092-044)
Guidelines:	Not indicated
Published:	Yes (Agricultural and Biological Chemistry, 1987, 51 (11), 3081-3086)
GLP:	No

Report:	CA 8/55, Yokota, T., Morita, M., Takahashi, N., 1983 c
Title:	6-DEOXOCASTASTERONE AND 6-DEOXODOLICHOSTERONE: PUTATIVE PRECURSORS FOR BRASSINOLIDE-RELATED STEROIDS FROM PHASEOLUS VULGARIS
Laboratory report / project number (Doc. No.)	Not applicable (092-074)
Guidelines:	Not indicated
Published:	Yes (Agricultural and Biological Chemistry, 1983, 47 (9), 2149-2151)
GLP:	No

Report:	CA 8/56, Yokota, T., Koba, S., Kim, S.K., Takatsuto, S., Ikekawa, N., Sakakibara, M., Okada, K., Mori, K., Takahashi, N., 1987
Title:	DIVERSE STRUCTURAL VARIATIONS OF THE BRASSINOSTEROIDS IN PHASEOLUS VULGARIS SEED
Laboratory report / project number (Doc. No.)	Not applicable (092-076)
Guidelines:	Not indicated
Published:	Yes (Agricultural and Biological Chemistry, 1987, 51 (6), 1625-1631)
GLP:	No

Report:	CA 8/57, Kim, S.-K., Yokota, T., Takahashi, N., 1987
Title:	25-METHYLDOLICHOSTERONE, A NEW BRASSINOSTEROID WITH A TERTIARY BUTYL GROUP FROM IMMATURE SEED OF PHASEOLUS VULGARIS
Laboratory report / project number (Doc. No.)	Not applicable (092-032)
Guidelines:	Not indicated
Published:	Yes (Agricultural and Biological Chemistry, 1987, 51 (8), 2303-2305)
GLP:	No

Report:	CA 8/58, Kim, T.-W., Park, S.-H., Han, K.-S., Choo, J., Lee, J.S., Hwang, S., Kim, S.-K., 2000 b
Title:	OCCURRENCE OF TEASTERONE AND TYPHASTEROL, AND THEIR ENZYMATIC CONVERSION IN PHASEOLUS VULGARIS
Laboratory report / project number (Doc. No.)	Not applicable (092-035)
Guidelines:	Not indicated
Published:	Yes (Bulletin-Korean Chemical Society, 2000, 21 (4), 373-374)
GLP:	No

Report:	CA 8/59, Kim, S.-K., 1991
Title:	NATURAL OCCURRENCES OF BRASSINOSTEROIDS
Laboratory report / project number (Doc. No.)	Not applicable (092-033)
Guidelines:	Not indicated
Published:	Yes (ACS Symposium series, 1991, 474, Chapter 3, 26-35)
GLP:	No

Report:	CA 8/60, Park, S.C., Kim, T.-W., Kim, S.-K., 2000
Title:	IDENTIFICATION OF BRASSINOSTEROIDS WITH 24R-METHYL IN IMMATURE SEEDS OF PHASEOLUS VULGARIS
Laboratory report / project number (Doc. No.)	Not applicable (092-043)
Guidelines:	Not indicated
Published:	Yes (Bulletin-Korean Chemical Society, 2000, 21 (12), 1274-1276)
GLP:	No

Report:	CA 8/61, Nomura, T., Nakayama, M., Reid, J.B., Takeuchi, Y., Yokota, T., 1997
Title:	BLOCKAGE OF BRASSINOSTEROID BIOSYNTHESIS AND SENSITIVITY CAUSES DWARFISM IN GARDEN PEA
Laboratory report / project number (Doc. No.)	Not applicable (092-038)
Guidelines:	Not indicated
Published:	Yes (Plant Physiology, 1997, 113, 31-37)
GLP:	No

Report:	CA 8/62, Nomura, T., Kitasaka, Y., Takatsuto, S., Reid, J.B., Fukami, M., Yokota, T., 1999
Title:	BRASSINOSTEROID/STEROL SYNTHESIS AND PLANT GROWTH AS AFFECTED BY IKA AND IKB MUTATIONS OF PEA
Laboratory report / project number (Doc. No.)	Not applicable (092-039)
Guidelines:	Not indicated
Published:	Yes (Plant Physiology, 1999, 119, 1517-1527)

GLP:	No

Report:	CA 8/63, Sondhi, N., Bhardwaj, R., Kaur, S., Kumar, N., Singh, B., 2008
Title:	ISOLATION OF 24-EPIBRASSINOLIDE FROM LEAVES OF AEGLE MARMELOS AND EVALUATION OF ITS ANTIGENOTOXITIY EMPLOYING ALLIUM CEPA CHROMOSOMAL ABERRATION ASSAY
Laboratory report / project number (Doc. No.)	Not applicable (092-154)
Guidelines:	Not indicated
Published:	Yes (Plant Growth Regulation, 2008, 54, 217-224; DOI: 10.1007/s10725-007-9242-7)
GLP:	No

Report:	CA 8/64, Motegi, C., Takatsuto, S., 1994
Title:	IDENTIFICATION OF BRASSINOLIDE AND CASTASTERONE IN THE POLLEN OF ORANGE (CITRUS SINENSIS OSBECK) BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY
Laboratory report / project number (Doc. No.)	Not applicable (092-037)
Guidelines:	Not indicated
Published:	Yes (Journal of Chromatography A, 1994, 658, 27-30)
GLP:	No

Report:	CA 8/65, Abe, H., Morishita, T., Uchiyama, M., Takatsuto, S., Ikekawa, N., 1984 a
Title:	A NEW BRASSINOLIDE-RELATED STEROID IN THE LEAVES OF THEA SINENSIS
Laboratory report / project number (Doc. No.)	Not applicable (092-003)
Guidelines:	Not indicated
Published:	Yes (Agricultural and Biological Chemistry, 1984, 48 (8), 2171-2172)
GLP:	No

Report:	CA 8/66, Gupta, D., Bhardwaj, R., Nagar, P.K., Kaur, S., 2004
Title:	ISOLATION AND CHARACTERIZATION OF BRASSINOSTEROIDS FROM LEAVES OF CAMELLIA SINENSIS (L.) O. KUNTZE
Laboratory report / project number (Doc. No.)	Not applicable (092-153)
Guidelines:	Not indicated
Published:	Yes (Plant Growth Regulation, 2004, 43, 97-100)
GLP:	No

Report:	CA 8/67, Choi, Y.-H., Inoue, T., Fujioka, S., Saimoto, H., Sakurai, A., 1993
Title:	IDENTIFICATION OF BRASSINOSTEROID-LIKE ACTIVE STUBSTANCES IN PLANT-CELL CULTURES
Laboratory report /	Not applicable (092-016)

project number (Doc. No.)	
Guidelines:	Not indicated
Published:	Yes (Bioscience, Biotechnology and Biochemistry, 1993, 57 (5), 860-861)
GLP:	No

Report:	CA 8/68, Fujioka, S., Inoue, T., Takatsuto, S., Yanagisawa, T., Yokota, T., Sakurai, A., 1995
Title:	IDENTIFICATION OF A NEW BRASSINOSTEROID, CATHASTERONE, IN CULTURED CELLS OF CATHARANTHUS ROSEUS AS A BIOSYNTHETIC PRECURSOR OF TEASTERONE
Laboratory report / project number (Doc. No.)	Not applicable (092-017)
Guidelines:	Not indicated
Published:	Yes (Bioscience, Biotechnology and Biochemistry, 1995, 59 (8), 1543-1547)
GLP:	No

Report:	CA 8/69, Park, K.-H., Saimoto, H., Nakagawa, S., Sakurai, A., Yokota, T., Takahashi, N., Syono, K., 1989
Title:	OCCURRENCE OF BRASSINOLIDE AND CASTASTERONE IN CROWN GALL CELLS OF CATHARANTHUS ROSEUS
Laboratory report / project number (Doc. No.)	Not applicable (092-045)
Guidelines:	Not indicated
Published:	Yes (Agricultural and Biological Chemistry, 1989, 53 (3), 805-811)
GLP:	No

Report:	CA 8/70, Suzuki, H., Fujioka, S., Takatsuto, S., Yokota, T., Murofushi, N., Sakurai, A., 1995
Title:	BIOSYNTHESIS OF BRASSINOSTEROIDS IN SEEDLINGS OF CATHARANTHUS ROSEUS, NICOTIANA TABACUM, AND ORYZA SATIVA
Laboratory report / project number (Doc. No.)	Not applicable (092-056)
Guidelines:	Not indicated
Published:	Yes (Bioscience, Biotechnology and Biochemistry, 1995, 59 (2), 168-172)
GLP:	No

Report:	CA 8/71, Yokota, T., Ogino, Y., Takahashi, N., Saimoto, H., Fujioka, S., Sakurai, A., 1990
Title:	BRASSINOLIDE IS BIOSYNTHESIZED FROM CASTASTERONE IN CATHARANTHUS ROSEUS CROWN GALL CELLS
Laboratory report / project number (Doc. No.)	Not applicable (092-077)
Guidelines:	Not indicated
Published:	Yes (Agricultural and Biological Chemistry, 1990, 54 (4), 1107-1108)

GLP:	No

Report:	CA 8/72, Takatsuto, S., Yokota, T., Omote, K., Gamor, K., Takahashi, N., 1989
Title:	IDENTIFICATION OF BRASSINOLIDE, CASTASTERONE AND NORCASTASTERONE (BRASSINONE) IN SUNFLOWER (HELIANTHUS ANNUUS L.) POLLEN
Laboratory report / project number (Doc. No.)	Not applicable (092-058)
Guidelines:	Not indicated
Published:	Yes (Agricultural and Biological Chemistry, 1989, 53 (8), 2177-2180)
GLP:	No

Report:	CA 8/73, Yamamoto, R., Fujioka, S., Demura, T., Takatsuto, S., Yoshida, S., Fukuda, H., 2001
Title:	BRASSINOSTEROID LEVELS INCREASE DRASTICALLY PRIOR TO MORPHOGENESIS OF TRACHEARY ELEMENTS
Laboratory report / project number (Doc. No.)	Not applicable (092-066)
Guidelines:	Not indicated
Published:	Yes (Plant Physiology, 2001, 125, 556-563)
GLP:	No

Report:	CA 8/74, Suzuki, Y., Yamaguchi, I., Takahasi, N., 1985
Title:	IDENTIFICATION OF CASTASTERONE AND BRASSINONE FROM IMMATURE SEEDS OF PHARBITIS PURPUREA
Laboratory report / project number (Doc. No.)	Not applicable (092-052)
Guidelines:	Not indicated
Published:	Yes (Agricultural and Biological Chemistry, 1985, 49 (1), 49-54)
GLP:	No

Report:	CA 8/75, Jang, M.-S., Han, K.-S., Kim, S.-K., 2000
Title:	IDENTIFICATION OF BRASSINOSTEROIDS AND THEIR BIOSYNTHETIC PRECURSORS FROM SEEDS OF PUMPKIN
Laboratory report / project number (Doc. No.)	Not applicable (092-028)
Guidelines:	Not indicated
Published:	Yes (Bulletin-Korean Chemical Society, 2000, 21 (2), 161-164)
GLP:	No

Report:	CA 8/76, Tripathi, S., Sharma, P., 2015
Title:	CHARACTERIZATION OF BRASSINOSTEROID ISOLATED FROM BACOPA MONNIERI L. AND THEIR FREE RADICAL SCAVENGING ACTIVITY
Laboratory report / project number (Doc. No.)	Not applicable (092-156)
Guidelines:	Not indicated
Published:	Yes (International Journal of Science and Research (IJSR), 2015, 4 (4), 2738-2742)
GLP:	No

Report:	CA 8/77, Yokota, T., Nomura, T., Nakayama, M., 1997 d
Title:	IDENTIFICATION OF BRASSINOSTEROIDS THAT APPEAR TO BE DERIVED FROM CAMPESTEROL AND CHOLESTEROL IN TOMATO SHOOTS
Laboratory report / project number (Doc. No.)	Not applicable (092-070)
Guidelines:	Not indicated
Published:	Yes (Plant & Cell Physiology, 1997, 38 (11), 1291-1294)
GLP:	No

Report:	CA 8/78, Bishop, G.J., Nomura, T., Yokota, T., Harrison, k., Noguchi, T., Fujioka, S., Takatsuto, S., Jones, J.D.G., Kamiya, Y., 1999
Title:	THE TOMATO DWARF ENZYME CATALYSES C-6 OXIDATION IN BRASSINOSTEROID BIOSYNTHESIS
Laboratory report / project number (Doc. No.)	Not applicable (092-014)
Guidelines:	Not indicated
Published:	Yes (Proceedings of the National Academy of Sciences, 1999, 96, 1761-1766)
GLP:	No

Report:	CA 8/79, Griffiths, P.G., Sasse, J.M., Yokota, T., Cameron, D.W., 1995
Title:	6-DEOXOTYPHASTEROL AND 3-DEHYDRO-6-DEOXOTEASTERONE, POSSIBLE PRECURSORS TO BRASSINOSTEROIDS IN THE POLLEN OF CUPRESSUS ARIZONICA
Laboratory report / project number (Doc. No.)	Not applicable (092-021)
Guidelines:	Not indicated
Published:	Yes (Bioscience, Biotechnology and Biochemistry, 1995, 59 (5), 956-959)
GLP:	No

Report:	CA 8/80, Takatsuto, S., Abe, H., Shimada, K., Nakayama, M., Yokota, T., 1996 a
Title:	IDENTIFICATION OF TEASTERONE AND 4-DESMETHYLSTEROLS IN THE SEEDS OF GINKGO BILOBA L.
Laboratory report / project number (Doc. No.)	Not applicable (092-061)
Guidelines:	Not indicated
Published:	Yes (Japan Oil Chemists' Society, 1996, 45 (12), 1349-1351)
GLP:	No

Report:	CA 8/81, Kim, S.-K., Abe, H., Anthony Little, C.H., Pharis, R.P., 1990
Title:	IDENTIFICATION OF TWO BRASSINOSTEROIDS FROM THE CAMBIAL REGION OF SCOTS PINE (PINUS SILVERSTRIS) BY GAS CHROMATOGRAPHY-MASS SPECTROMETRY, AFTER DETECTION USING A DWARF RICE LAMINA INCLINATION BIOASSAY
Laboratory report / project number (Doc. No.)	Not applicable (092-031)
Guidelines:	Not indicated
Published:	Yes (Plant Physiology, 1990, 94, 1709-1713)
GLP:	No

Report:	CA 8/82, Yokota, T., Arima, M., Takahashi, N., Takatsuto, S., Ikekawa, N., Takematsu, T., 1983 a
Title:	2-DEOXYCASTASTERONE, A NEW BRASSINOLIDE-RELATED BIOACTIVE STEROID FROM PINUS POLLEN
Laboratory report / project number (Doc. No.)	Not applicable (092-071)
Guidelines:	Not indicated
Published:	Yes (Agricultural and Biological Chemistry, 1983, 47 (10), 2419-2420)
GLP:	No

Report:	CA 8/83, Yokota, T., Higuchi, K., Takahashi, N., Kamuro, Y., Watanabe, T., Takatsuto, S., 1998
Title:	IDENTIFICATION OF BRASSINOSTEROIDS WITH EPIMERIZED SUBSTITUENTS AND / OR THE 23-OXO GROUP IN POLLEN AND ANTHERS OF JAPANESE CEDAR
Laboratory report / project number (Doc. No.)	Not applicable (092-068)
Guidelines:	Not indicated
Published:	Yes (Bioscience, Biotechnology and Biochemistry, 1998, 62 (3), 526-531)
GLP:	No

Report:	CA 8/84, Watanabe, T., Yokota, T., Shibata, K., Nomura, T., Seto, H., Takatsuto, S., 2000
Title:	CRYPTOLIDE, A NEW BRASSINOLIDE CATABOLITE WITH A 23-OXO GROUP FROM JAPANESE CEDAR POLLEN/ANTHER AND ITS SYNTHESIS
Laboratory report / project number (Doc. No.)	Not applicable (092-065)
Guidelines:	Not indicated
Published:	Yes (Journal of Chemical Research (S), 2000, 18-19)
GLP:	No

Report:	CA 8/85, Park, S.-H., Han, K.-S., Kim, T.-W., Shim, J.-K., Takatsuto, S., Yokota, T., Kim, S.-K., 1999
Title:	IN VIVO AND IN VITRO CONVERSION OF TEASTERONE TO TYPHASTEROL IN CULTURED CELLS OF MARCHANTIA POLYMORPHA
Laboratory report / project number (Doc. No.)	Not applicable (092-042)
Guidelines:	Not indicated
Published:	Yes (Plant & Cell Physiology, 1999, 40 (9), 955-960)
GLP:	No

Report:	CA 8/86, Yokota, T., Ohnishi, T., Shibata, K., Asahina, M., Nomura, T., Fujita, T., Ishizaki, K., Kohchi, T., 2017
Title:	OCCURRENCE OF BRASSINOSTEROIDS IN NON-FLOWERING LAND PLANTS, LIVERWORT, MOSS, LYCOPHYTE AND FERN
Laboratory report / project number (Doc. No.)	Not applicable (092-069)
Guidelines:	Not indicated
Published:	Yes (Phytochemistry, 2017, xxx, 1-10; doi: 10.1016/j.phytochem.2016.12.020)
GLP:	No

Report:	CA 8/87, Stirk, W.A., Balint, P., Tarkowska, D., Novak, O., Strnad, M., Oerdoeg, V., van Staden, J., 2013
Title:	HORMONE PROFILES IN MICROALGAE: GIBBERELLINS AND

	BRASSINOSTEROIDS
Laboratory report / project number (Doc. No.)	Not applicable (092-051)
Guidelines:	Not indicated
Published:	Yes (Plant Physiology and Biochemistry, 2013, 70, 348-353; doi: 10.1016/j.plaphy.2013.05.037)
GLP:	No

Report:	CA 8/88, Bajguz, A., 2009
Title:	ISOLATION AND CHARACTERIZATION OF BRASSINOSTEROIDS FROM ALGAL CULTURES OF CHLORELLA VULGARIS BEIJERINCK (TREBOUXIOPHYCEAE)
Laboratory report / project number (Doc. No.)	Not applicable (092-013)
Guidelines:	Not indicated
Published:	Yes (Journal of Plant Physiology, 2009, 166, 1946-1949; doi:10.1016/j.jplph.2009.05.003)
GLP:	No

Report:	CA 8/89, Hamdy, A.-H. A., Aboutabl, E.A., Sameer, S., Hussein, A.A., Diaz-Marrero, A.R., Darias, J., Cueto, M., 2009
Title:	3-KETO-22-EPI-28-NOR-CATHASTERONE, A BRASSINOSTEROID-RELATED METABOLITE FROM CYSTOSEIRA MYRICA
Laboratory report / project number (Doc. No.)	Not applicable (092-023)
Guidelines:	Not indicated
Published:	Yes (Steroids, 2009, 74, 927-930; doi: 10.1016/j.steroids.2009.06.008)
GLP:	No

Report:	CA 8/90, Tsavkelova, E.A., Klimova, S.Y., Cherdyntseva, T.A., Netrusov, A.I., 2006
Title:	HORMONES AND HORMONE-LIKE SUBSTANCES OF MICROORGANISMS: A REVIEW
Laboratory report / project number (Doc. No.)	Not applicable (092-064)
Guidelines:	Not indicated
Published:	Yes (Applied Biochemistry and Microbiology, 2006, 42 (3), 229-235)
GLP:	No

Report:	CA 8/91, Bajguz, A., Hayat, S., 2009
Title:	EFFECTS OF BRASSINOSTEROIDS ON THE PLANT RESPONSES TO ENVIRONMENTAL STRESSES
Laboratory report / project number (Doc. No.)	Not applicable (092-133)

Guidelines:	Not indicated
Published:	Yes (Plant Physiology and Biochemistry, 2009, 47, 1-8; doi:10.1016/j.plaphy.2008.10.002)
GLP:	No

Report:	CA 8/92, Eremina, M., Unterholzner, S.J., Rathnayake, A., Castellano, M., Khan, M., Kugler, K.G., May, S.T., Mayer, K.F., Rozhon, W., Poppenberger, B., 2016
Title:	BRASSINOSTEROIDS PARTICIPATE IN THE CONTROL OF BASAL AND ACQUIRED FREEZING TOLERANCE OF PLANTS
Laboratory report / project number (Doc. No.)	Not applicable (092-136)
Guidelines:	Not indicated
Published:	Yes (Proceedings of the National Academy of Sciences, 2016, 113 (40), E5982-E5991)
GLP:	No

Report:	CA 8/93, Aremu, A.O., Stirk, W.A., Kulkarni, M.G., Tarkowska, D., Tureckova, V., Gruz, J., Subrtova, M., Pencik, A., Novak, O., Dolezal, K., Strnad, M., Van Staden, J., 2015
Title:	EVIDENCE OF PHYTOHORMONES AND PHENOLIC ACIDS VARIABILITY IN GARDEN-WASTE-DERIVED VERMICOMPOST LEACHATE, A WELL-KNOWN PLANT GROWTH STIMULANT
Laboratory report / project number (Doc. No.)	Not applicable (092-158)
Guidelines:	Not indicated
Published:	Yes (Plant Growth Regulation, 2015, 75 (2), 483-492; DOI: 10.1007/s10725-014-0011-0)
GLP:	No

Report:	CA 8/94, Badri, D.V., Vivanco, J.M., 2009
Title:	REGULATION AND FUNCTION OF ROOT EXUDATES
Laboratory report / project number (Doc. No.)	Not applicable (092-012)
Guidelines:	Not indicated
Published:	Yes (Plant, Cell and Environment, 2009, 32, 666-681; doi: 10.1111/j.1365-3040.2009.01926.x)
GLP:	No

Report:	CA 8/95, Hassett, J.P., Fred Lee, G., Lee, F.G., 1977
Title:	STEROLS IN NATURAL WATER AND SEDIMENT
Laboratory report / project number (Doc. No.)	Not applicable (092-168)
Guidelines:	Not indicated
Published:	Yes (Water Research, 1977, 11, 983-989)
GLP:	No

Report:	CA 8/96, Mudge, S.M., Joao A.F. Bebianno, M., East, J.A., Barreira, L.A., 1999
Title:	STEROLS IN THE RIA FORMOSA LAGOON, PORTUGAL
Laboratory report / project number (Doc. No.)	Not applicable (092-169)
Guidelines:	Not indicated
Published:	Yes (Water Research, 1999, 33 (4), 1038-1048)
GLP:	No

Report:	CA 8/97, Nishikawa, N., Toyama, S., Shida, A., Futatsuya, F., 1994
Title:	THE UPTAKE AND THE TRANSPORT OF 14C-LABELED EPIBRASSINOLIDE IN INTACT SEEDLINGS OF CUCUMBER AND WHEAT
Laboratory report / project number (Doc. No.)	Not applicable (092-088)
Guidelines:	Not indicated
Published:	Yes (Journal of Plant Research, 1994, 107, 125-130)
GLP:	No

Report:	CA 8/98, Mekhalfi, M., Avilan, L., Lebrun, R., Botebol, H., Gontero, B., 2012
Title:	CONSEQUENCES OF THE PRESENCE OF 24-EPIBRASSINOLIDE, ON CULTURES OF A DIATOM, ASTERIONELLA FORMOSA
Laboratory report / project number (Doc. No.)	Not applicable (092-109)
Guidelines:	Not indicated
Published:	Yes (Biochimie, 2012, 94, 1213-1220; doi: 10.1016/j.biochi.2012.02.011)
GLP:	No

Report:	CA 8/99, Vorbrodt, H.-M., Adam, G., Porzel, A., Hoerhold, C., Daenhardt, S., Boehme, K.-H., 1991
Title:	MICROBIAL DEGRADATION OF 2 ALPHA, 3 ALPHA-DIHYDROXY-5 ALPHA-CHOLESTAN-6-ONE BY MYCOBACTERIUM VACCAE
Laboratory report / project number (Doc. No.)	Not applicable (092-157)
Guidelines:	Not indicated
Published:	Yes (Steroids, 1991, 56, 586-588)
GLP:	No

Report:	CA 8/100, Voigt, B., Porzel, A., Naumann, H., Hoerhold-Schubert, C., Adam, G., 1993
Title:	HYDROXYLATION OF THE NATIVE BRASSINOSTEROIDS 24-EPICASTASTERONE AND 24-EPIBRASSINOLIDE BY THE FUNGUS CUNNINGHAMELLA ECHINULATA
Laboratory report / project number	Not applicable (092-096)

(Doc. No.)	
Guidelines:	Not indicated
Published:	Yes (Steroids, 1993, 58, 320-323)
GLP:	No

Report:	CA 8/101, Saygideger, S., Deniz, F., 2008
Title:	EFFECT OF 24-EPIBRASSINOLIDE ON BIOMASS, GROWTH AND FREE PROLINE CONCENTRATION IN SPIRULINA PLATENSIS (CYNOPHYTA) UNDER NaCl STRESS
Laboratory report / project number (Doc. No.)	Not applicable (092-176)
Guidelines:	Not indicated
Published:	Yes (Plant Growth Regulation, 2008, 56, 219-223; DOI: 10.1007/s10725-008-9310-7)
GLP:	No

Report:	CA 8/102, Asari, S., Tarkowska, D., Rolcik, J., Novak, O., Palmero, D.V., Bejai, S., Meijer, J., 2017
Title:	ANALYSIS OF PLANT GROWTH-PROMOTING PROPERTIES OF BACILLUS AMYLOLIQUEFACIENS UCMB5113 USING ARABIDOPSIS THALIANA AS HOST PLANT
Laboratory report / project number (Doc. No.)	Not applicable (092-181)
Guidelines:	Not indicated
Published:	Yes (Planta, 2017, 245, 15-30; DOI: 10.1007/s00425-016-2580-9)
GLP:	No

Report:	CA 8.1.1.1/01, Aremu, A.O., Stirk, W.A., Kulkarni, M.G., Tarkowska, D., Tureckova, V., Gruz, J., Subrtova, M., Pencik, A., Novak, O., Dolezal, K., Strnad, M., Van Staden, J., 2015
Title:	EVIDENCE OF PHYTOHORMONES AND PHENOLIC ACIDS VARIABILITY IN GARDEN-WASTE-DERIVED VERMICOMPOST LEACHATE, A WELL-KNOWN PLANT GROWTH STIMULANT
Laboratory report / project number (Doc. No.)	Not applicable (092-158)
Guidelines:	Not indicated
Published:	Yes (Plant Growth Regulation, 2015, 75 (2), 483-492; DOI: 10.1007/s10725-014-0011-0)
GLP:	No

Report:	CA 8.1.1.1/02, Hassett, J.P., Fred Lee, G., 1977
Title:	STEROLS IN NATURAL WATER AND SEDIMENT
Laboratory report / project number (Doc. No.)	Not applicable (092-168)
Guidelines:	

Published:	Yes (Water Research, 1977, 11, 983-989)
GLP:	No

Report:	CA 8.1.1.1/03, Mudge, S.M., Joao A.F. Bebianno, M., East, J.A., Barreira, L.A., 1999
Title:	STEROLS IN THE RIA FORMOSA LAGOON, PORTUGAL
Laboratory report / project number (Doc. No.)	Not applicable (092-169)
Guidelines:	Not indicated
Published:	Yes (Water Research, 1999, 33 (4), 1038-1048)
GLP:	No

Report:	CA 8.1.1.1/04, Turfitt, G.E., 1942
Title:	THE MICROBIOLOGICAL DEGRADATION OF STEROIDS - 1. THE STEROL CONTENT OF SOILS
Laboratory report / project number (Doc. No.)	Not applicable (092-159)
Guidelines:	Not indicated
Published:	Yes (Biochemical Journal, 1943, 37, 115–117)
GLP:	No

Report:	CA 8.1.1.1/05, Heumann, S., Schlichting, A., Boettcher, J., Leinweber, P., 2011
Title:	STEROLS IN SOIL ORGANIC MATTER IN RELATION TO NITROGEN MINERALIZATION IN SANDY ARABLE SOILS
Laboratory report / project number (Doc. No.)	Not applicable (092-160)
Guidelines:	Not indicated
Published:	Yes (Journal of Plant Nutrition and Soil Science, 2011, 174, 576-586; DOI: 10.1002/jpln.200900273)
GLP:	No

Report:	CA 8.1.1.1/06, Kutschera, U., Wang, Z.-Y., 2012
Title:	BRASSINOSTEROID ACTION IN FLOWERING PLANTS: A DARWINIAN PERSPECTIVE
Laboratory report / project number (Doc. No.)	Not applicable (092-036)
Guidelines:	
Published:	Yes (Journal of Experimental Botany, 2012, 63 (10), 3511-3522; doi:10.1093/jxb/ers065)
GLP:	No

Report:	CA 8.1.1.1/07, Bajguz, A., 2007
Title:	METABOLISM OF BRASSINOSTEROIDS IN PLANTS
Laboratory report / project number (Doc. No.)	Not applicable (092-079)
Guidelines:	Not indicated
Published:	Yes (Plant Physiology and Biochemistry, 2007, 45, 95-107; doi:10.1016/j.plaphy.2007.01.002)
GLP:	No

Report:	CA 8.1.1.1/08, Fujioka, S., Yokota, T., 2003
Title:	BIOSYNTHESIS AND METABOLISM OF BRASSINOSTEROIDS
Laboratory report / project number (Doc. No.)	Not applicable (092-082)
Guidelines:	Not indicated
Published:	Yes (Annual Review of Plant Biology, 2003, 54, 137-164)
GLP:	No

Report:	CA 8.1.1.1/09, Hai, T., Schneider, B., Adam, G., 1995
Title:	METABOLIC CONVERSION OF 24-EPI-BRASSINOLIDE INTO PENTAHYDROXYLATED BRASSINOSTEROID GLUCOSIDES IN TOMATO CELL CULTURES
Laboratory report / project number (Doc. No.)	Not applicable (092-083)
Guidelines:	Not indicated
Published:	Yes (Phytochemistry, 1995, 40 (2), 443-448)
GLP:	No

Report:	CA 8.1.1.1/10, Schneider, B., Kolbe, A., Porzel, A., Adam, G., 1994
Title:	A METABOLITE OF 24-EPI-BRASSINOLIDE IN CELL SUSPENSION CULTURES OF LYCOPERSICON ESCULENTUM
Laboratory report / project number (Doc. No.)	Not applicable (092-092)
Guidelines:	Not indicated
Published:	Yes (Phytochemistry, 1994, 36 (2), 319-321)
GLP:	No

Report:	CA 8.1.1.1/11, Kolbe, A., Schneider, B., Porzel, A., Schmidt, J., Adam, G., 1995
Title:	ACYL-CONJUGATED METABOLITES OF BRASSINOSTEROIDS IN CELL SUSPENSION CULTURES OF ORNITHOPUS SATIVUS
Laboratory report / project number (Doc. No.)	Not applicable (092-085)
Guidelines:	Not indicated
Published:	Yes (Phytochemistry, 1995, 38 (3), 633-636)
GLP:	No

Report:	CA 8.1.1.1/12, Nishikawa, N., Shida, A., Toyama, S., 1995
Title:	METABOLISM OF 14C-LABELED EPIBRASSINOLIDE IN INTACT SEEDLINGS OF CUCUMBER AND WHEAT
Laboratory report / project number (Doc. No.)	Not applicable (092-087)
Guidelines:	Not indicated
Published:	Yes (Journal of Plant Research, 1995, 108, 65-69)
GLP:	No

Report:	CA 8.1.1.1/13, Kolbe, A., Schneider, B., Porzel, A., Adam, G., 1996
Title:	METABOLISM OF 24-EPI-CASTASTERONE AND 24-EPI-BRASSINOLIDE IN CELL SUSPENSION CULTURES OF ORNITHOPUS SATIVUS
Laboratory report / project number (Doc. No.)	Not applicable (092-086)
Guidelines:	Not indicated
Published:	Yes (Phytochemistry, 1996, 41 (1), 163-167)
GLP:	No

Report:	CA 8.1.1.1/14, Voigt, B., Porzel, A., Naumann, H., Hoerhold-Schubert, C., Adam, G., 1993
Title:	HYDROXYLATION OF THE NATIVE BRASSINOSTEROIDS 24-EPICASTASTERONE AND 24-EPIBRASSINOLIDE BY THE FUNGUS CUNNINGHAMELLA ECHINULATA
Laboratory report / project number (Doc. No.)	Not applicable (092-096)
Guidelines:	Not indicated
Published:	Yes (Steroids, 1993, 58, 320-323)
GLP:	No

Report:	CA 8.1.1.1/15, Winter, J., Schneider, B., Strack, D., Adam, G., 1997
Title:	ROLE OF A CYTOCHROME P450-DEPENDENT MONOOXYGENASE IN THE HYDROXYLATION OF 24-EPI-BRASSINOLIDE
Laboratory report / project number (Doc. No.)	Not applicable (092-097)
Guidelines:	Not indicated
Published:	Yes (Phytochemistry, 1997, 45 (2), 233-237)
GLP:	No

Report:	CA 8.1.1.1/16, Khripach, V., Zhabinskii, V., De Groot, A., 2000
Title:	TWENTY YEARS OF BRASSINOSTEROIDS: STEROIDAL PLANT HORMONES WARRANT BETTER CROPS FOR THE XXI CENTURY
Laboratory report / project number (Doc. No.)	Not applicable (092-029)
Guidelines:	
Published:	Yes (Annals of Botany, 2000, 86, 441-447; doi:10.1006/anbo.2000.1227)
GLP:	No

Report:	CA 8.1.2.1.1/02, Xingwu, D., Yun, X., Gang, L., Xiaofei, G., Hongmci, L., 2010
Title:	FIELD CAPACITY IN BLACK SOIL REGION, NORTHEAST CHINA
Laboratory report / project number (Doc. No.)	Not applicable (092-171)
Guidelines:	Not indicated
Published:	Yes (Chinese Geographical Science, 2010, 20 (5), 406-413; DOI: 10.1007/s11769-010-0414-4)
GLP:	No

Report:	CA 8.1.2.1.1/03, Anonymous, 2005
Title:	SOIL ATLAS OF EUROPE
Laboratory report / project number (Doc. No.)	Not applicable (092-17 0)
Guidelines:	Not indicated
Published:	Yes (European Commision, Joint Research Centre, 2005, ISBN 92-894-8120-X)
GLP:	No

Report:	CA 8.1.2.1.1/04, Kong, X., Lal, R., Li, B., Liu, H., Li, K., Feng, G., Zhang, Q., Zhang, B., 2014
Title:	FERTILIZER INTENSIFICATION AND ITS IMPACTS IN CHINA'S HHH PLAINS
Laboratory report / project number (Doc. No.)	Not applicable (092-180)
Guidelines:	Not indicated
Published:	Yes (Advances in Agronomy, 2014, 125, 135-169; DOI: 10.1016/B978-0-12-800137-0.00004-2)
GLP:	No

Report:	CA 8.1.2.1.1/05, Zhang, M., He, Z., Wilson, M.J., 2004
Title:	CHEMICAL AND PHYSICAL CHARACTERISTICS OF RED SOILS FROM ZHEJIANG PROVINE, SOUTHERN CHINA
Laboratory report / project number (Doc. No.)	Not applicable (092-184)
Guidelines:	Not indicated
Published:	Yes (The Red Soils of China, 2004, 63-87)
GLP:	No

Report:	CA 8.1.2.2/01, Aremu, A.O., Stirk, W.A., Kulkarni, M.G., Tarkowska, D., Tureckova, V., Gruz, J., Subrtova, M., Pencik, A., Novak, O., Dolezal, K., Strnad, M., Van Staden, J., 2015
Title:	EVIDENCE OF PHYTOHORMONES AND PHENOLIC ACIDS VARIABILITY IN GARDEN-WASTE-DERIVED VERMICOMPOST LEACHATE, A WELL-KNOWN PLANT GROWTH STIMULANT
Laboratory report / project number (Doc. No.)	Not applicable (092-158)
Guidelines:	Not indicated
Published:	Yes (Plant Growth Regulation, 2015, 75 (2), 483-492; DOI: 10.1007/s10725-014-0011-0)
GLP:	No

Report:	CA 8.1.2.2/02, Hassett, J.P., Fred Lee, G., 1977
Title:	STEROLS IN NATURAL WATER AND SEDIMENT
Laboratory report / project number (Doc. No.)	Not applicable (092-168)
Guidelines:	Not indicated
Published:	Yes (Water Research, 1977, 11, 983-989)
GLP:	No

Report:	CA 8.1.2.2/03, Mudge, S.M., Joao A.F. Bebianno, M., East, J.A., Barreira, L.A., 1999
Title:	STEROLS IN THE RIA FORMOSA LAGOON, PORTUGAL
Laboratory report / project number (Doc. No.)	Not applicable (092-169)
Guidelines:	Not indicated
Published:	Yes (Water Research, 1999, 33 (4), 1038-1048)
GLP:	No

Report:	CA 8.1.3.1/01, Aremu, A.O., Stirk, W.A., Kulkarni, M.G., Tarkowska, D., Tureckova, V., Gruz, J., Subrtova, M., Pencik, A., Novak, O., Dolezal, K., Strnad, M., Van Staden, J., 2015
Title:	EVIDENCE OF PHYTOHORMONES AND PHENOLIC ACIDS VARIABILITY IN GARDEN-WASTE-DERIVED VERMICOMPOST

	LEACHATE, A WELL-KNOWN PLANT GROWTH STIMULANT
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Guidelines:	Not indicated
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GLP:	No

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Guidelines:	Not indicated
Published:	Yes (Water Research, 1999, 33 (4), 1038-1048)
GLP:	No

Report:	CA 8.1.3.1/04, Briggs, G.G., Bromilow, R.H., Evans, A.A., 1982
Title:	RELATIONSHIPS BETWEEN LIPOPHILICITY AND ROOT UPTAKE AND TRANSLOCATION OF NON-IONISED CHEMICALS BY BARLEY
Laboratory report / project number (Doc. No.)	Not applicable (092-172)
Guidelines:	Not indicated
Published:	Yes (Pest Management Science, 1982, 13, 495-504)
GLP:	No

Report:	CA 8.1.3.1/05, Ryan, J.A., Bell, R.M., Davidson, J.M., O'Connor, G.A., 1988
Title:	PLANT UPTAKE OF NON-IONIC ORGANIC CHEMICALS FROM SOILS
Laboratory report / project number (Doc. No.)	Not applicable (092-173)
Guidelines:	Not indicated
Published:	Yes (Chemosphere, 1988, 17 (12), 2299-2323)
GLP:	No

Report:	CA 8.1.3.1/06,Yokota, T., Higuchi, K., Kosaka, Y., Takahashi, N., 1992
Title:	TRANSPORT AND METABOLISM OF BRASSINOSTEROIDS IN RICE

Laboratory report / project number (Doc. No.)	Not applicable (092-098)
Guidelines:	Not indicated
Published:	Yes (Progress in Plant Growth Regulation, 1992, 13, 298-305)
GLP:	No

Report:	CA 8.1.3.1/07,Nishikawa, N., Toyama, S., Shida, A., Futatsuya, F., 1994
Title:	THE UPTAKE AND THE TRANSPORT OF 14C-LABELED EPIBRASSINOLIDE IN INTACT SEEDLINGS OF CUCUMBER AND WHEAT
Laboratory report / project number (Doc. No.)	Not applicable (092-088)
Guidelines:	Not indicated
Published:	Yes (Journal of Plant Research, 1994, 107, 125-130)
GLP:	No

Report:	CA 8.1.4/01, Zhu, J.-Y., Sae-Seaw, J., Wang, Z.-Y., 2013
Title:	BRASSINOSTEROID SIGNALLING
Laboratory report / project number (Doc. No.)	Not applicable (092-165)
Guidelines:	Not indicated
Published:	Yes (Development, 2013, 140(8), 1615-1620; doi: 10.1242/dev.060590)
GLP:	No

Report:	CA 8.1.4/02, Aremu, A.O., Stirk, W.A., Kulkarni, M.G., Tarkowska, D., Tureckova, V., Gruz, J., Subrtova, M., Pencik, A., Novak, O., Dolezal, K., Strnad, M., Van Staden, J., 2015
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Laboratory report / project number (Doc. No.)	Not applicable (092-158)
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Laboratory report / project number (Doc. No.)	Not applicable (092-169)
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Published:	Yes (Water Research, 1999, 33 (4), 1038-1048)
GLP:	No

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Report:	CA 8.1.4/06, Ryan, J.A., Bell, R.M., Davidson, J.M., O'Connor, G.A., 1988
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GLP:	No

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Guidelines:	Not indicated
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GLP:	No

Report:	CA 8.1.4/08, Nishikawa, N., Toyama, S., Shida, A., Futatsuya, F., 1994
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	EPIBRASSINOLIDE IN INTACT SEEDLINGS OF CUCUMBER AND WHEAT
Laboratory report / project number (Doc. No.)	Not applicable (092-088)
Guidelines:	Not indicated
Published:	Yes (Journal of Plant Research, 1994, 107, 125-130)
GLP:	No

Report:	CA 8.2/01, Takatsuto, S., Abe, H., Gamoah, K., 1990 a
Title:	EVIDENCE FOR BRASSINOSTEROIDS IN STROBILUS OF EQUISETUM ARVENSE L.
Laboratory report / project number (Doc. No.)	Not applicable (092-059)
Guidelines:	Not indicated
Published:	Yes (Agricultural and Biological Chemistry, 1990, 54 (4), 1057-1059)
GLP:	No

Report:	CA 8.2/02, Aremu, A.O., Stirk, W.A., Kulkarni, M.G., Tarkowska, D., Tureckova, V., Gruz, J., Subrtova, M., Pencik, A., Novak, O., Dolezal, K., Strnad, M., Van Staden, J., 2015
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GLP:	No

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Laboratory report / project number (Doc. No.)	Not applicable (092-169)
Guidelines:	Not indicated
Published:	Yes (Water Research, 1999, 33 (4), 1038-1048)
GLP:	No

Report:	CA 8.2.1.1/02, Mekhalfi, M., Avilan, L., Lebrun, R., Botebol, H., Gontero, B., 2012
Title:	CONSEQUENCES OF THE PRESENCE OF 24-EPIBRASSINOLIDE, ON CULTURES OF A DIATOM, ASTERIONELLA FORMOSA
Laboratory report / project number (Doc. No.)	Not applicable (092-109)
Guidelines:	Not indicated
Published:	Yes (Biochimie, 2012, 94, 1213-1220; doi: 10.1016/j.biochi.2012.02.011)
GLP:	No

Report:	CA 8.2.2/01, Zhu, J.-Y., Sae-Seaw, J., Wang, Z.-Y., 2013
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Report:	CA 8.2.2/02, Aremu, A.O., Stirk, W.A., Kulkarni, M.G., Tarkowska, D., Tureckova, V., Gruz, J., Subrtova, M., Pencik, A., Novak, O., Dolezal, K., Strnad, M., Van Staden, J., 2015
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Guidelines:	Not indicated
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Laboratory report / project number (Doc. No.)	Not applicable (092-169)
Guidelines:	Not indicated
Published:	Yes (Water Research, 1999, 33 (4), 1038-1048)
GLP:	No

Report:	CA 8.2.2/05, Voigt, B., Porzel, A., Naumann, H., Hoerhold-Schubert, C., Adam, G., 1993
Title:	HYDROXYLATION OF THE NATIVE BRASSINOSTEROIDS 24-EPICASTASTERONE AND 24-EPIBRASSINOLIDE BY THE FUNGUS CUNNINGHAMELLA ECHINULATA
Laboratory report / project number (Doc. No.)	Not applicable (092-096)
Guidelines:	Not indicated
Published:	Yes (Steroids, 1993, 58, 320-323)
GLP:	No

Report:	CA 8.2.2/06, Vorbrodt, H.-M., Adam, G., Porzel, A., Hoerhold, C., Daenhardt, S., Boehme, K.-H., 1991
Title:	MICROBIAL DEGRADATION OF 2 ALPHA, 3 ALPHA-DIHYDROXY-5 ALPHA-CHOLESTAN-6-ONE BY MYCOBACTERIUM VACCAE
Laboratory report / project number (Doc. No.)	Not applicable (092-157)
Guidelines:	Not indicated
Published:	Yes (Steroids, 1991, 56, 586-588)
GLP:	No

Report:	CA 8.2.2/07, Gartz, J., Adam, G., Vorbrodt, H.-M., 1990
Title:	GROWTH-PROMOTING EFFECT OF A BRASSINOSTEROID IN MYCELIAL CULTURES OF THE FUNGUS PSILOCYBE CUBENSIS
Laboratory report / project number (Doc. No.)	Not applicable (092-174)
Guidelines:	Not indicated
Published:	Yes (Naturwissenschaften, 1990, 77, 388-389)

GLP:	NoNot indicated

Report:	CA 8.2.2/08, Ding, Y., Zhu, Z., Zhao, J., Nie, Y., Zhang, Y., Sheng, J., Meng, D., Mao, H., Tang, X., 2016
Title:	EFFECTS OF POSTHARVEST BRASSINOLIDE TREATMENT ON THE METABOLISM OF WHITE BUTTON MUSHROOM (AGARICUS BISPORUS) IN RELATION TO DEVELOPMENT OF BROWNING DURING STORAGE
Laboratory report / project number (Doc. No.)	Not applicable (092-175)
Guidelines:	Not indicated
Published:	Yes (Food and Bioprocess Technology, 2016; DOI: 10.1007/s11947-016-1722-1)
GLP:	No

Report:	CA 8.2.2/09, Khripach, V.A., Zhabinskii, V.N., Khripach, N.B., 2003
Title:	CHAPTER 9 - NEW PRACTICAL ASPECTS OF BRASSINOSTEROIDS AND RESULTS OF THEIR TEN-YEAR AGRICULTURAL USE IN RUSSIA AND BELARUS
Laboratory report / project number (Doc. No.)	Not applicable (092-179)
Guidelines:	Not indicated
Published:	Yes (Brassinosteroids, Chapter 9, 2003, 189-230)
GLP:	No

Report:	CA 8.2.2/10, Saygideger, S., Deniz, F., 2008
Title:	EFFECT OF 24-EPIBRASSINOLIDE ON BIOMASS, GROWTH AND FREE PROLINE CONCENTRATION IN SPIRULINA PLATENSIS (CYNOPHYTA) UNDER NaCl STRESS
Laboratory report / project number (Doc. No.)	Not applicable (092-176)
Guidelines:	Not indicated
Published:	Yes (Plant Growth Regulation, 2008, 56, 219-223; DOI: 10.1007/s10725-008-9310-7)
GLP:	No

Report:	CA 8.2.2/11, Mekhalfi, M., Avilan, L., Lebrun, R., Botebol, H., Gontero, B., 2012
Title:	CONSEQUENCES OF THE PRESENCE OF 24-EPIBRASSINOLIDE, ON CULTURES OF A DIATOM, ASTERIONELLA FORMOSA
Laboratory report / project number (Doc. No.)	Not applicable (092-109)
Guidelines:	Not indicated
Published:	Yes (Biochimie, 2012, 94, 1213-1220; doi: 10.1016/j.biochi.2012.02.011)
GLP:	No

Report:	CA 8.2.2/12, Bajguz, A., 2011
Title:	SUPPRESSION OF CHLORELLA VULGARIS GROWTH BY CADMIUM, LEAD, AND COPPER STRESS AND ITS RESTORATION BY ENDOGENOUS BRASSINOLIDE
Laboratory report / project number (Doc. No.)	Not applicable (092-103)
Guidelines:	Not indicated
Published:	Yes (Archives of Environmental Contamination and Toxicology, 2011, 60, 406-416; DOI 10.1007/s00244-010-9551-0)
GLP:	No

Report:	CA 8.2.2.1/01, Aremu, A.O., Stirk, W.A., Kulkarni, M.G., Tarkowska, D., Tureckova, V., Gruz, J., Subrtova, M., Pencik, A., Novak, O., Dolezal, K., Strnad, M., Van Staden, J., 2015
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Guidelines:	Not indicated
Published:	Yes (Water Research, 1977, 11, 983-989)
GLP:	No

Report:	CA 8.2.2.1/03, Mudge, S.M., Joao A.F. Bebianno, M., East, J.A., Barreira, L.A., 1999
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Laboratory report / project number (Doc. No.)	Not applicable (092-169)
Guidelines:	Not indicated
Published:	Yes (Water Research, 1999, 33 (4), 1038-1048)
GLP:	No

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Title:	CONSEQUENCES OF THE PRESENCE OF 24-EPIBRASSINOLIDE, ON CULTURES OF A DIATOM, ASTERIONELLA FORMOSA

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Guidelines:	Not indicated
Published:	Yes (Biochimie, 2012, 94, 1213-1220; doi: 10.1016/j.biochi.2012.02.011)
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Report:	CA 8.4/01, Aremu, A.O., Stirk, W.A., Kulkarni, M.G., Tarkowska, D., Tureckova, V., Gruz, J., Subrtova, M., Pencik, A., Novak, O., Dolezal, K., Strnad, M., Van Staden, J., 2015
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GLP:	No

Report:	CA 8.4/02, Hassett, J.P., Fred Lee, G., 1977
Title:	STEROLS IN NATURAL WATER AND SEDIMENT
Laboratory report / project number (Doc. No.)	Not applicable (092-168)
Guidelines:	
Published:	Yes (Water Research, 1977, 11, 983-989)
GLP:	No

Report:	CA 8.4/03, Mudge, S.M., Joao A.F. Bebianno, M., East, J.A., Barreira, L.A., 1999
Title:	STEROLS IN THE RIA FORMOSA LAGOON, PORTUGAL
Laboratory report / project number (Doc. No.)	Not applicable (092-169)
Guidelines:	Not indicated
Published:	Yes (Water Research, 1999, 33 (4), 1038-1048)
GLP:	No

Report:	CA 8.5/01, Hassett, J.P., Fred Lee, G., 1977
Title:	STEROLS IN NATURAL WATER AND SEDIMENT
Laboratory report / project number (Doc. No.)	Not applicable (092-168)
Guidelines:	Not indicated
Published:	Yes (Water Research, 1977, 11, 983-989)
GLP:	No

Appendix I

The applicant submitted an introduction to brassinosteroids in support with the dossier of 24-Epibrassinolide. This general information was not evaluated in detail by RMS because it is not deemed necessary for the evaluation. Nonetheless it is presented in this Appendix I for completeness (in *italics*) and the full references cited in the introduction can be found at the end:

Brassinosteroids, including 24-Epibrassinolide are naturally occurring, plant growth promoting molecules, present in higher plants, lower plants, including algae, mosses, the "living fossil" Equisetum as well as some fungi (Takatsuto et al., 1990a, Table A-1). Brassinosteroids are present in all plant organs such as pollen, anthers, seeds, leaves, stems, roots, flowers, grains and fruits with the highest concentrations found in pollen, seeds and fruits (Zhu et al., 2013) and considered an obligatory plant constituent. Pollen and immature seeds show contents of Brassinosteroids in a range of 0.001 – 6400 µg/kg fresh weight, while shoots and leaves usually show lower concentrations of 0.001 – 100 µg/kg fresh weight. Fruits, e,g. apples contain 10-35 µg/kg fresh weight (Table A-2). The concentration of Brassinosteroids in plants is regulated by a complex system of feedback pathways (e.g. Saini et al., 2015) and Brassinosteroids are constantly synthesised, metabolised, activated and inactivated depending on the plant's needs as well as environmental cues. The concentrations of Brassinosteroids are continuously fluctuating - spatially and temporally: in a single plant, different concentrations can be measured simultaneously in different plant organs, cell structures and cells as well as in the same location at different times (e.g. Symons et al., 2008).

Brassinosteroids represent ubiquitous, phylogenetically ancient phytohormones that promote growth in land plants as well as in green freshwater algae. According to Kutschera and Wang (2012), Brassinosteroids may have evolved in the Pre-Cambrian, at a time during the evolution of life on earth, when the split between uni- and multicellular green algae (which later gave rise to the embryophytes) had not yet occurred.

24-Epibrassinolide was first synthesized in 1979 (Thompson et al., 1979). Ten years later the natural occurrence of 24-Epibrassinolide in the plant kingdom was demonstrated by isolation and detection of 24-Epibrassinolide in Vicia faba pollen (Ikekawa et al., 1988) for the first time. Isolation of 24-Epibrassinolide and other Brassinosteroids, respectively, from natural materials is a complicated and expensive process. Therefore, 24-Epibrassinolide is chemically synthesized, identical to the naturally occurring 24-Epibrassinolide and is considered a "natural-identical synthesized molecule".

Brassinosteroids, which belong to the class of polyhdroxysteroids, can be divided into free as well as conjugated signal molecules. They are classified by their alkyl-substitutions in the side chain, as C_{27}, C_{28} or C_{29} Brassinosteroids (Table A-1).

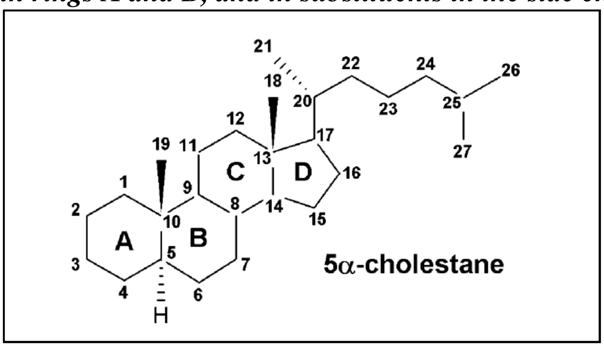
Table A-1: Division of free brassinosteroids according to number of carbon in structure and different types of B-ring and substituents in the A-ring brassinolide (BL), Castasterone (CS), Cathasterone (CT), Dolicholide (DL), Dolichosterone (DS), Dehydroteasterone (DT), Methyl (Me), Secasterone (SE), Teasterone (TE), Typhasterol (TY)

No. of carbon	*Type of B-ring*	*Substituent in A-ring*	*Representative(s)*
C27	*7-Oxalactone*	*C(2α,3α)-OH*	*28-norBL*
	6-Oxo	*C(2α,3α)-OH*	*28-norCS*
		C3α-OH	*28-norTY*
	6-Deoxo	*C(2α,3α)-OH*	*6-deoxo-28-norCS*
		C3α-OH	*6-deoxo-28-norTY, 3-epi-6-deoxo-28-norCT*
		C3β-OH	*6-deoxo-28-norTE, 6-deoxo-28-norCT*
		C3-oxo group	*3-dehydro-6-deoxo-28-norTE, 3-keto-22-epi-28-norCT*
C28	*7-Oxalactone*	*C(2α,3α)-OH*	*BL, 24-epiBL, 23-dehydroBL, DL*

		C(2α,3β)-OH	*3-epi-23-dehydroBL, 3-epiBL*
		C(2β,3α)-OH	*2-epi-23-dehydroBL*
		C(2β,3β)-OH	*2,3-diepi-23-dehydroBL*
		C3α-OH	*2-deoxyBL, 7-oxTY*
		C3β-OH	*7-oxTE*
	6-Oxo	*C(2α,3α)-OH*	*CS, 24-epiCS, DS*
		C(2α,3β)-OH	*3-epiCS, 3,24-diepiCS*
		C(2β,3α)-OH	*2-epiCS*
		C(2β,3β)-OH	*2,3-diepiCS*
		C(1β,2α,3α)-OH	*1β-OH-CS*
		C(1α,2α,3β)-OH	*3-epi-1α-OH-CS*
		C3α-OH	*TY*
		C3β-OH	*TE, CT*
		C3-oxo group	*3-DT (3-dehydroTE)*
		C(2β,3β)-epoxide	*SE, 24-epiSE*
		C(2α,3α)-epoxide	*2,3-diepiSE*
		$\Delta^{2,3}$	*Secasterol*
	6-Deoxo	*C(2α,3α)-OH*	*6-deoxoCS, 6-deoxo-24-epiCS, 6-deoxoDS*
		C(2α,3β)-OH	*3-epi-6-deoxoCS*
		C3α-OH	*6-deoxoTY, 3-epi-6-deoxoCT*
		C3β-OH	*6-deoxoTE, 6-deoxoCT*
		C3-oxo group	*6-deoxo-3DT (3-dehydro-6-deoxoTE)*
	6-Hydroxy	*C(2α,3α)-OH*	*6α-OH-CS*
C29	*7-Oxalactone*	*C(2α,3α)-OH*	*28-homoBL, 28-homoDL*
	6-Oxo	*C(2α,3α)-OH*	*28-homoCS, 28-homoDS, 25-MeDS, 25-MeCS*
		C(2β,3α)-OH	*2-epi-25-MeDS, 2-epi-25-MeCS*
		C(2β,3β)-OH	*2,3-diepi-25-MeDS, 2,3-diepi-25-MeCS*
		C3α-OH	*28-homoTY, 2-deoxy-25-MeDS*
		C3β-OH	*28-homoTE, 3-epi-2-deoxy-25-MeDS*
	6-Deoxo	*C(2α,3α)-OH*	*6-deoxo-28-homoDS, 6-deoxo-25-MeDS*

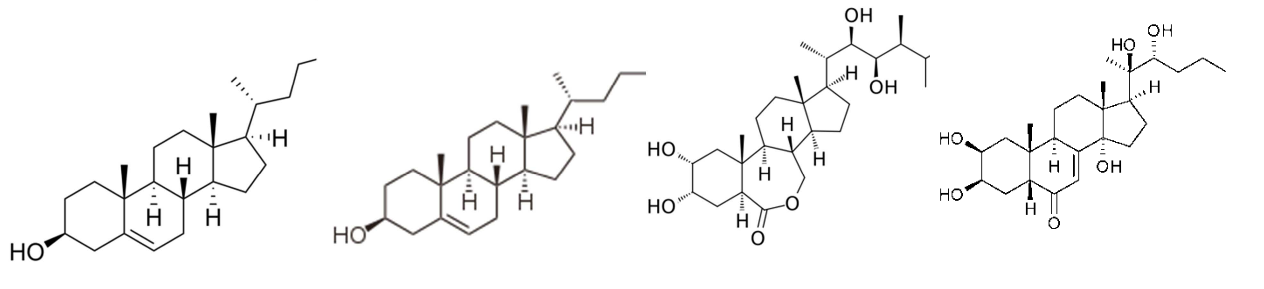
Most Brassinosteroids, including the physiologically most important C_{28} brassinolides, are synthesized by the precursor campesterol via a common 5α-cholestane skeleton. Structural variations are synthesized by differences in orientation of the oxygenated functions in rings A and B, and by different substituents in the side chain (Bajguz, 2011, Figure A-1).

Figure A-1: 5α-cholestane as the skeleton of Brassinosteroid synthesis. Structural differences occur in rings A and B, and in substituents in the side chain C_{20} – C_{27}.



Campesterol (Figure A-2a) derives from 5α-cholestane and, with its attached alcohol group, chemically represents a sterol, like e.g. the animal sterol cholesterol (Figure A-2b) or the insect derived molting hormone ecdysterone (Figure A-2d). Campesterol exhibits a double bond from carbon 5 to carbon 6 and therefore can be defined as a Δ^5 sterol. During synthesis of 24-Epibrassinolide, campesterol becomes fully saturated (Δ^0) by creation of an additional carbonyl bond and attachment of hydroxyl groups to the side chains. Therefore, 24-Epibrassinolide represents no longer a sterol but a stanol as, per definition, stanols are saturated or reduced sterols that share structural similarities with the campesterol/cholesterol skeleton. Due to the fact that alkenes (double bonds between carbons), as found in other plant steroids, display a chemical bond of higher reactivity, 24-Epibrassinolide has to be differentiated concerning its *structural chemistry for the absence of an alkene group. This difference for example minimizes stanol absorption in the mammalian intestines (Bajguz, 2011).*

Figure A-2: Structure of the (a) precursor Campesterol, (b) the animal derived molecule Cholesterol, (c) the active substance 24-Epibrassinolide, and (d) the insect hormone Ecdysterone.



(a) Campesterol *(b) Cholesterol* *(c) brassinolide* *(d) Ecdysteron*

24-Epibrassinolide belongs, besides 28-Homobrassinolide and Brassinolide, to the most biologically active Brassinosteroids, all three of them having similar chemical structures. 28-homobrassinolide and 24-Epibrassinolide differ from Brassinolide by the substituent in the side chain at C24 or by its configuration at C24, respectively (Khripach et al., 2000). All three act in low concentrations between 0.1 – 0.001 ppm (Ikekawa and Zhao, 1991).

Table A-2 is a summary table based on open literature and without any claim to completeness. It is to be expected that Brassinosteroids are also ubiquitous distributed in organisms not included in this table.

Table A-2: Natural occurrence and concentrations of Brassinosteroids in higher and lower plants, fungi, processed and unprocessed foodstuffs

Family/Species	*Examined part*	*Brassino-steroid*[1]	*content μg/kg fr. wt.*[2]	*References*
Monocotyledons				
Arecaceae				
Date palm (Phoenix dactylifera)	*pollen*	*24-epiCS*	*unspecified*	*Zaki et al., 1993*[3]
Gramineae				
Perennial ryegrass (Lolium perenne L.)	*pollen*	*BR (1)*	*0.001*	*Taylor et al., 1993*[3]
Rice (Oryza sativa L.)	*shoot*	*BL*	*unspecified*	*Abe et al., 1984b; Abe 1991*[3]
	shoot	*CS*	*0.014*	*Abe et al., 1984b; Abe 1991*[3]
	shoot	*BR (1)*	*0.008*	*Abe et al., 1984b; Abe 1991*[3]
	bran	*BR (3)*	*unspecified*	*Abe et al., 1995a*
	seeds	*CS, BR (2)*	*unspecified*	*Park et al., 1994b*
	grains	*24-epiBL*	*216*	*Khripach et al., 2013*
	grains	*BL*	*29*	*Khripach et al., 2013*
	grains	*28-homoBL*	*4.4*	*Khripach et al., 2013*
Canary grass (Phalaris canariensis)	*seeds*	*CS*	*5*	*Shimada et al.,1996*[3]
	seeds	*BR (1)*	*0.7*	*Shimada et al.,1996*[3]
Common wheat (Triticum aestivum L.)	*grain*	*CS, BR (4)*	*unspecified*	*Yokota et al, 1994*
Rye (Secale cereal)	*seeds*	*CS, BR (4)*	*unspecified*	*Schmidt et al., 1995b*[3]
	leaves	*BR (3)*	*0.02-0.052*	*Antonchick et al., 2003*[3]
	roots	*BR (2)*	*0.032-0.107*	*Antonchick et al., 2003*[3]
Maize (Zea mays L.) - dent corn	*pollen*	*CS*	*120*	*Suzuki et al., 1986*
	pollen	*BR (2)*	*4.1-6.6*	*Suzuki et al., 1986*
	roots	*CS*	*0.3*	*Kim et al., 2000a*

Family/Species	*Examined part*	*Brassino-steroid*[1]	*content µg/kg fr. wt.*[2]	*References*
Maize (Zea mays L.) - sweet corn	*pollen*	*CS*	*27.2*	*Gamoh et al., 1990*[3]
	pollen	*BR (2)*	*16.9-18.3*	*Gamoh et al., 1990*[3]
Liliaceae				
Asian fawnlily (Erythronium japonicum Decne)	*pollen, anther*	*BR (1)*	*5*	*Yasuta et al., 1995*
Lilium elegans Thunb.	*pollen*	*CS*	*10-50*	*Suzuki et al., 1994b ; Yasuta et al., 1995*
Liliaceae				
	pollen	*BL*	*1-5*	*Suzuki et al., 1994b ; Yasuta et al., 1995*
	pollen	*BR (2)*	*1-50*	*Suzuki et al., 1994b ; Yasuta et al., 1995*
Lilium longiflorum Thunb.	*pollen*	*BR (1)*	*3180*	*Abe, 1991*[3] *; Abe et al., 1994 ; Asakawa et al.,1994, 1996 ; Soeno et al., 2000*
Lilium longiflorum Thunb.	*anther*	*BL, CS*	*unspecified*	*Abe, 1991*[3] *; Abe et al., 1994 ; Asakawa et al.,1994, 1996 ; Soeno et al., 2000*
	anther	*BR (5)*	*20-2440*	*Abe, 1991*[3] *; Abe et al., 1994 ; Asakawa et al.,1994, 1996 ; Soeno et al., 2000*
Garden tulip (Tulipa gesneriana L.)	*pollen*	*BR (1)*	*unspecified*	*Abe, 1991*[3]
Typhaceae				
Broadleaf cattail (Typha latifolia)	*pollen*	*BR (2)*	*68*	*Schneider et al., 1983*[3]*; Abe, 1991*[3]
Dicotyledons – Apetalae				
Betulaceae				
Common alder (Alnus glutinosa (L.))	*pollen*	*BL, CS*	*unspecified*	*Plattner et al., 1986*
Cannabaceae				
Hemp (Cannabis sativa L.)	*seeds*	*CS*	*600*	*Takatsuto et al., 1996b*
	seeds	*BR (1)*	*1800*	*Takatsuto et al., 1996b*
Caryophyllaceae				
Gypsophilla perfoliata L.	*seeds*	*24-epiBL*	*unspecified*	*Schmidt et al., 1996*
Sticky catchfly (Lychnis viscaria L.)	*seeds*	*24-epiCS, BR (1)*	*unspecified*	*Friebe et al., 1999*[3]
Chenophyllaceae				
Beet (Beta vulgaris L.)	*seeds*	*CS, 24-epiCS*	*unspecified*	*Schmidt et al., 1994*[3]
Fagaceae				
Japanese chestnut (Castanea crenata Sieb. Et Zucc.)	*galls*	*BL*	*0.001-12*	*Yokota et al., 1982a, Ikeda et al., 1983, Ikekawa & Takatsuto, 1984*
	galls	*CS*	*0.011-11.43*	*Yokota et al., 1982a, Ikeda et al., 1983, Ikekawa & Takatsuto, 1984*
	galls	*BR (2)*	*0.011-26*	*Yokota et al., 1982a, Ikeda et al., 1983, Ikekawa & Takatsuto, 1984*
	shoot	*BR (1)*	*15-30*	*Arima et al., 1984*
	leaves	*CS*	*2-6*	*Arima et al., 1984*
Polygonaceae				
Common buckwheat (Fagopyrum esculentum Moench)	*pollen*	*BL*	*5*	*Takatsuto et al., 1990b*
	pollen	*CS*	*7.1*	*Takatsuto et al., 1990b*
	grains	*24-epiBL*	*378*	*Khripach et al, 2013*

Family/Species	*Examined part*	*Brassino-steroid[1]*	*content µg/kg fr. wt.[2]*	*References*
	grains	*BL*	*40*	*Khripach et al, 2013*
	grains	*28-homoBL*	*8.1*	*Khripach et al, 2013*
Pieplant (Rheum rhabarbarum L.)	*panicles*	*BL, CS, 24-epiCS*	*unspecified*	*Schmidt et al., 1995a[3]*
Dicotyledons – Chloripetalae				
Apiaceae				
Asian pennywort (Centella asiatica)	*leaves*	*CS*	*unspecified*	*Sondhi et al., 2010*
Celery (Apium graveolens L.)	*seeds*	*BR (1)*	*unspecified*	*Schmidt et al., 1995c[3]*
Wild carrot (Daucus carota ssp. Sativus L.)	*seeds*	*BL, CS, 24-epiCS*	*unspecified*	*Schmidt et al., 1998[3]*
	root	*24-epiBL*	*0.43*	*Khripach et al., 2013*
	root	*BL*	*1.5*	*Khripach et al., 2013*
	root	*28-homoBR*	*0.83*	*Khripach et al., 2013*
	root	*24-epiCS*	*0.23*	*Khripach et al., 2013*
	whole plant	*24-epiBL*	*0.745*	*Swaczynová et al., 2007*
	whole plant	*BL*	*0.644*	*Swaczynová et al., 2007*
	whole plant	*CS*	*0.316*	*Swaczynová et al., 2007*
	whole plant	*24-epiCS*	*0.642-1.19*	*Swaczynová et al., 2007*
Brassicaceae				
Arabidopsis thaliana (L.) Heynh.	*shoot*	*BL*	*0.04*	*Fujioka et al., 1996, 1997, 2000a[3]; Nomura et al., 2001*
	shoot	*CS*	*0.75*	*Fujioka et al., 1996, 1997, 2000a[3]; Nomura et al., 2001*
	shoot	*BR (9)*	*0.025-1.96*	*Fujioka et al., 1996, 1997, 2000a[3]; Nomura et al., 2001*
	20-days-old shoots	*BR (5)*	*0.1-0.79*	*Bancos et al., 2002*
	20-days-old shoots	*CS*	*0.15*	*Bancos et al., 2002*
	seeds	*24-epiBL*	*0.22*	*Fujioka et al., 1998[3]*
	seeds	*BL*	*0.5-1.9*	*Fujioka et al., 1998[3]*
	seeds	*CS*	*0.4-5*	*Fujioka et al., 1998[3]*
	seeds	*BR (4)*	*0.5-5.4*	*Fujioka et al., 1998[3]*
	seeds	*24-epiBL*	*0.22*	*Schmidt et al., 1997*
	seeds	*CS*	*0.36*	*Schmidt et al., 1997*
	root callus	*BL, BR (1)*	*unspecified*	*Konstantinova et al., 2001[3]*
	20-days-old roots	*BR (5)*	*0.09-1.8*	*Bancos et al., 2002*
	20-days-old roots	*CS*	*0.035*	*Bancos et al., 2002*
	seedlings	*BR (10)*	*unspecified*	*Choe et al., 2001 ; Fujioka et al. 2002*
	whole plant	*24-epiBL*	*3.634-4.566*	*Swaczynová et al., 2007*
	whole plant	*BL*	*1.245*	*Swaczynová et al., 2007*
	whole plant	*CS*	*0.562*	*Swaczynová et al., 2007*
	apical shoot	*BR (6)*	*0.03-7.93*	*Shimada et al., 2003*
	apical shoot	*CS*	*2.02*	*Shimada et al., 2003*
	stem	*BR (5)*	*0.14-2.64*	*Shimada et al., 2003*
	stem	*CS*	*0.40*	*Shimada et al., 2003*
	cauline leaves	*BR (5)*	*0.11-4.33*	*Shimada et al., 2003*
	cauline leaves	*CS*	*0.31*	*Shimada et al., 2003*
	rosette leaves	*BR (5)*	*0.06-2.85*	*Shimada et al., 2003*
	rosette leaves	*CS*	*0.13*	*Shimada et al., 2003*

Family/Species	Examined part	Brassino-steroid[1]	content µg/kg fr. wt.[2]	References
	silique	BR (5)	0.36-8.89	Shimada et al., 2003
	silique	CS	0.94	Shimada et al., 2003
Chinese Cabbage (Brassica campestris var. pekinensis L.)	seeds	BL	940	Abe et al., 1982, 1983[3]; Ikekawa et al., 1984[3]
	seeds	CS	1600	Abe et al., 1982, 1983[3]; Ikekawa et al., 1984[3]
	seeds	28-homoCS	130	Abe et al., 1982, 1983[3]; Ikekawa et al., 1984[3]
	seeds	BR (2)	780-1300	Abe et al., 1982, 1983[3]; Ikekawa et al., 1984[3]
	immature seeds and sheaths	BL	0.0094	Ikekawa & Takatsuto, 1984
	immature seeds and sheaths	CS	0.0016	Ikekawa & Takatsuto, 1984
	immature seeds and sheaths	BR (3)	0.0013-0.00078	Ikekawa & Takatsuto, 1984
Indian mustard (Brassica juncea L.)	fresh leaves	24-epiBL	unspecified	Kanwar et al., 2013
Oilseed rape (Brassica napus L.)	pollen	BL	100	Grove et al., 1979
	breaking wall pollen	24-epiBL	628	Pan et al., 2012
	pollen	BL	101.664	Swaczynová et al., 2007
	pollen	CS	12.166	Swaczynová et al., 2007
Radish (Raphanus sativus L.)	seeds	BL	0.3	Schmidt et al., 1991[3], 1993b[3]
	seeds	CS	0.8	Schmidt et al., 1991[3], 1993b[3]
	seeds	BR (2)	unspecified	Schmidt et al., 1991[3], 1993b[3]
	germinated seeds	BL	0.45	Schmidt et al., 1991[3]
	germinated seeds	CS	0.4	Schmidt et al., 1991[3]
Fabaceae				
Lablab bean (Dolichos lablab L.)	seeds	BR (4)	12-160	Baba et al., 1983; Yokota et al., 1982b[3], 1983b, 1984
	seeds	BL, CS, BR (2)	unspecified	Baba et al., 1983; Yokota et al., 1982b[3], 1983b, 1984
Dolichos lablab	immature seeds	Homodolicholide	0.353	Yokota et al., 1983b
False acacia (Robinia pseudo-acacia)	pollen	CS, BR (2)	unspecified	Abe et al., 1995b
Broad bean (Vicia faba L.)	pollen	24-epiBL	5	Park et al., 1987; Ikekawa et al., 1988
	pollen	BL	190	Park et al., 1987; Ikekawa et al., 1988
	pollen	CS, BR (1)	unspecified	Park et al., 1987; Ikekawa et al., 1988
	pollen	BL	181	Gamoh et al., 1989[3]
	pollen	CS	134	Gamoh et al., 1989[3]
	pollen	BR (2)	537-628	Gamoh et al., 1989[3]
Serradella (Ornithopus sativus Brot.)	seeds	CS	5	Schmidt et al., 1993a[3]
	seeds	24-epiCS	25	Schmidt et al., 1993a[3]
	shoot	CS, 24-epiCS, BR (3)	unspecified	Spengler et al., 1995[3]
Common bean (Phaseolus vulgaris L.)	seeds	24-epiCS, BL, CS, BR (22)	unspecified	Yokota et al., 1983c, 1987 ; Kim et al., 1987, 1988[3], 2000b ; Kim, 1991 ; Park et al., 2000
	10-day-old seedlings	24-epiBL	<0.346	Swaczynová et al., 2007

Family/Species	*Examined part*	*Brassino-steroid[1]*	*content µg/kg fr. wt.[2]*	*References*
	10-day-old seedlings	*BL*	*0.471*	*Swaczynová et al., 2007*
	10-day-old seedlings	*CS*	*0.967*	*Swaczynová et al., 2007*
Goa bean (Psophocarpus tetragonolobus (Stickm.) DC.)	*seeds*	*BL, CS, BR (2)*	*unspecified*	*Takatsuto, 1994[3]*
Pea (Pisum sativum L.)	*seeds*	*BL, CS, BR (3)*	*unspecified*	*Yokota et al., 1996[3]*
	shoot	*BL*	*0.2-0.8*	*Nomura et al., 1997, 1999, 2001*
	shoot	*CS*	*0.4-2.4*	*Nomura et al., 1997, 1999, 2001*
	shoot	*BR (6)*	*0.047-5.2*	*Nomura et al., 1997, 1999, 2001*
	15- days-old shoots	*BR (5)*	*0.073-11.7*	*Bancos et al., 2002*
	15- days-old shoots	*CS*	*0.69*	*Bancos et al., 2002*
	shoots (36 d old)	*BL*	*0.164*	*Nomura et al., 1997*
	shoots (36 d old)	*CS*	*0.355*	*Nomura et al., 1997*
	shoots (36 d old)	*BR (1)*	*3.133*	*Nomura et al., 1997*
	6 months old plants	*BL*	*0.84*	*Nomura et al., 1997*
	6 months old plants	*CS*	*2.36*	*Nomura et al., 1997*
	6 months old plants	*BR (1)*	*0.995*	*Nomura et al., 1997*
	49-d-old shoots	*CS*	*0.491*	*Nomura et al., 1999*
	49-d-old shoots	*BR (7)*	*0.02-2.937*	*Nomura et al., 1999*
	15- days-old roots	*BR (6)*	*0.002-5.1*	*Bancos et al., 2002*
	15- days-old roots	*BL*	*0.024*	*Bancos et al., 2002*
	15- days-old roots	*CS*	*0.038*	*Bancos et al., 2002*
Hamamelidaceae				
Distylium racemosum Sieb. Et Zucc.	*galls*	*CS*	*2500*	*Ikekawa et al., 1984[3]*
	galls	*BR (1)*	*5*	*Ikekawa et al., 1984[3]*
	leaves	*BL*	*0.023*	*Ikekawa et al., 1984[3], Abe et al., 1994*
	leaves	*CS*	*0.13*	*Ikekawa et al., 1984[3], Abe et al., 1994*
	leaves	*BR(4)*	*0.016-0.16*	*Ikekawa et al., 1984[3], Abe et al., 1994*
Myrtaceae				
Eucalyptus calophylla R. Br.	*pollen*	*BL*	*unspecified*	*Takatsuto, 1994[3]*
Eucalyptus marginata Sn.	*pollen*	*BR (1)*	*unspecified*	*Takatsuto, 1994[3]*
Rosaceae				
Loquat (Eriobotyra japonica (Thunb.) Lindl.)	*flower, buds*	*CS*	*unspecified*	*Takatsuto, 1994[3]*
Apple (Malus domestica)	*fruit*	*24-epiBL*	*27*	*Khripach et al, 2013*
	fruit	*BL*	*35*	*Khripach et al, 2013*
	fruit	*28-homoBL*	*10*	*Khripach et al, 2013*
Rutaceae				
Bael tree (Aegle marmelos Corr.)	*leaves*	*24-epiBL*	*unspecidied*	*Sondhi et al., 2008*
Satsuma orange (Citrus unshiu Marcov.)	*pollen*	*BL, CS, BR (2)*	*unspecified*	*Abe, 1991[3]*

Family/Species	*Examined part*	*Brassino-steroid[1]*	*content µg/kg fr. wt.[2]*	*References*
Orange (Citrus sinensis Osbeck)	*pollen*	*BL*	*36.2*	*Motegi et al., 1994*
	pollen	*CS*	*29.4*	*Motegi et al., 1994*
Theaceae				
Chinese Tea (Thea sinensis L.)	*leaves*	*BL*	*0.006*	*Abe et al. 1983[3], 1984a; Morishita et al., 1983[3]; Ikekawa et al., 1984[3]*
	leaves	*CS*	*0.1*	*Abe et al. 1983[3], 1984a; Morishita et al., 1983[3]; Ikekawa et al., 1984[3]*
	leaves	*BR (4)*	*<0.001-0.06*	*Abe et al. 1983[3], 1984a; Morishita et al., 1983[3]; Ikekawa et al., 1984[3]*
	seeds	*BR (6)*	*unspecified*	*Kaur et al., 2002[3]*
Green tea	*leaves*	*24-epiBL*	*100*	*Khripach et al, 2013, Gupta et al., 2004*
	leaves	*BL*	*0.0046*	*Ikekawa & Takatsuto, 1984*
	leaves	*CS*	*0.11*	*Ikekawa & Takatsuto, 1984*
	leaves	*BR (6)*	*0.002*	*Ikekawa & Takatsuto, 1984, Gupta et al., 2004*
Dicotyledons – Sympetalae				
Apocynaceae				
Catharanthus roseus G. Don.	*culture cells*	*BL*	*0.4-8.7*	*Choi et al., 1993, 1996[3], 1997[3] ; Fujioka et al., 1995, 2000b[3] ; Park et al., 1989 ; Suzuki et al., 1993[3], 1994a[3], c, 1995 ; Yokota et al., 1990 ; Choe et al., 2001 ; Fujioka et al., 2002*
	culture cells	*CS*	*0.6-4.5*	*Choi et al., 1993, 1996[3], 1997[3] ; Fujioka et al., 1995, 2000b[3] ; Park et al., 1989 ; Suzuki et al., 1993[3], 1994a[3], c, 1995 ; Yokota et al., 1990 ; Choe et al., 2001 ; Fujioka et al., 2002*
	culture cells	*BR (17)*	*0.047-30*	*Choi et al., 1993, 1996[3], 1997[3] ; Fujioka et al., 1995, 2000b[3] ; Park et al., 1989 ; Suzuki et al., 1993[3], 1994a[3], c, 1995 ; Yokota et al., 1990 ; Choe et al., 2001 ; Fujioka et al., 2002*
Asteraceae				
Common sunflower (Helianthus anuus L.)	*pollen*	*BL*	*106*	*Takatsuto et al., 1989*
	pollen	*CS*	*21*	*Takatsuto et al., 1989*
	pollen	*BR (1)*	*65*	*Takatsuto et al., 1989*
	breaking wall pollen	*24-epiBL*	*1930*	*Pan et al., 2012*
Solidago altissima L.	*shoot*	*BL*	*unspecified*	*Takatsuto, 1994[3]*
Zinnia elegans L.	*culture cells*	*CS, BR (4)*	*unspecified*	*Yamamoto et al., 2001*
Boraginaceae				
Echium plantagineum L.	*pollen*	*BL*	*unspecified*	*Takatsuto, 1994[3]*
Convolvulaceae				
Pharbitis purpurea Voigt	*seeds*	*CS*	*1.1*	*Suzuki et al., 1985*
	seeds	*BR (1)*	*0.2*	*Suzuki et al., 1985*
Cucurbitaceae				
Cucurbita moschata Duch.	*seeds*	*BL, CS*	*unspecified*	*Jang et al., 2000*
Lamiaceae				
Perilla frutescens (L.) Britt.	*seeds*	*CS*	*unspecified*	*Park et al., 1994b*

Family/Species	*Examined part*	*Brassino-steroid*[1]	*content µg/kg fr. wt.*[2]	*References*
Plantaginaceae				
Coastal water hyssop (Bacopa monnieri L.)	*Fresh leaves*	*24-epiBL*	*unspecified*	*Tripathi & Sharma, 2015*
Rubiaceae				
Coffee (Coffea arabica)	*bean*	*24-epiBL*	*30*	*Khripach et al., 2013*
	bean	*BL*	*250*	*Khripach et al., 2013*
	bean	*28-homoBL*	*23*	*Khripach et al., 2013*
Solanaceae				
Tobacco (Nicotiana tabacum L.)	*culture cells*	*CS*	*unspecified*	*Park et al., 1994b*
Tomato (Lycopersicum esculentum Mill.)	*shoot*	*CS*	*0.2*	*Yokota et al., 1997d*
	shoot	*BR (2)*	*0.03-1.7*	*Yokota et al., 1997d*
	shoot (dwarf mutant)	*BL*	*<0.001*	*Bishop et al., 1999*
	shoot (dwarf mutant)	*CS*	*0.2*	*Bishop et al., 1999*
	shoot (dwarf mutant)	*BR (10)*	*<0.001-52*	*Bishop et al., 1999*
	36- days-old shoots	*BR (5)*	*0.016-0.64*	*Bancos et al., 2002*
	36- days-old shoots	*CS*	*0.14*	*Bancos et al., 2002*
	36- days-old roots	*BR (5)*	*0.062-2.8*	*Bancos et al., 2002*
	36- days-old roots	*CS*	*0.011*	*Bancos et al., 2002*
Potato (Solanum tuberosum)	*tuber*	*24-epiBL*	*37.5*	*Khripach et al., 2013*
	tuber	*BL*	*10*	*Khripach et al., 2013*
	tuber	*28-homoBL*	*1.5*	*Khripach et al., 2013*
	tuber	*Epi-CS*	*1.7*	*Khripach et al., 2013*
Gymnosperms				
Cupressaceae				
Cupressus arizonica Greene	*pollen*	*BL*	*<1*	*Griffiths et al., 1995*
	pollen	*CS*	*1000*	*Griffiths et al., 1995*
	pollen	*BR (7)*	*2-6400*	*Griffiths et al., 1995*
Ginkgoaceae				
Ginkgo biloba L.	*seeds*	*BR (1)*	*15*	*Takatsuto et al., 1996a*
Pinaceae				
Picea sitchensis Trantv. ex Mey	*shoot*	*CS*	*5*	*Yokota et al., 1985*[3]
	shoot	*BR (1)*	*7*	*Yokota et al., 1985*[3]
Pinus silvestris L.	*cambial region*	*BL, CS*	*unspecified*	*Kim et al., 1990*
Pinus thunbergii Parl.	*pollen*	*BR (1)*	*89.5*	*Yokota et al., 1983a*
Taxodiaceae				
Crypromeria japonica D. Don.	*pollen, anther*	*28-homoBL, BR (8)*	*unspecified*	*Yokota et al., 1998, Watanabe et al., 2000*
Lower plants				
Athyriaceae				
Black lady fern (Deparia japonica)	*fertile frond*	*CS*	*0.008*	*Yokota et al., 2017*
	fertile frond	*BR (7)*	*0.013-4.867*	*Yokota et al., 2017*
Asian common ladyfern (Athyrium yokoscense)	*reproductive frond*	*CS*	*0.002*	*Yokota et al., 2017*
	reproductive frond	*BR (6)*	*0.073-4.807*	*Yokota et al., 2017*

Family/Species	*Examined part*	*Brassino-steroid*[1]	*content µg/kg fr. wt.*[2]	*References*
Dennstaedtiaceae				
Eagle fern (Pteridium aquilinum)	*vegetative frond*	*CS*	*0.003*	*Yokota et al., 2017*
	vegetative frond	*BR (7)*	*0.021-1.873*	*Yokota et al., 2017*
Dryopteridaceae				
Wood fern (Dryopteris crassirhizoma)	*fertile frond*	*CS*	*0.024*	*Yokota et al., 2017*
	fertile frond	*BR (3)*	*0.019-0.802*	*Yokota et al., 2017*
Autumn fern (Dryopteris erythrosora)	*reproductive shoot*	*CS*	*0.005*	*Yokota et al., 2017*
	reproductive shoot	*BR (6)*	*0.008-20.87*	*Yokota et al., 2017*
Cyrtomium laetevirens	*reproductive shoot*	*CS*	*0.002*	*Yokota et al., 2017*
	reproductive shoot	*BR (5)*	*0.006-3.172*	*Yokota et al., 2017*
Equisetaceae				
Field Horsetail (Equisetum arvense L.)	*whole plant*	*CS*	*0.17*	*Takatsuto et al., 1990a*
	whole plant	*BR (3)*	*0.15-0.75*	*Takatsuto et al., 1990a*
	shoot	*CS*	*0.003-0.008*	*Yokota et al., 2017*
	shoot	*BR (8)*	*0.02-2*	*Yokota et al., 2017*
Funariaceae				
Spreading earth-moss (Physcomitrella patens)	*protonema*	*CS*	*0.004*	*Yokota et al., 2017*
	protonema	*BR (8)*	*0.008-1.122*	*Yokota et al., 2017*
Lygodiaceae				
Vine-like fern (Lygodium japonicum)	*vegetative frond*	*CS*	*0.016*	*Yokota et al., 2017*
	vegetative frond	*BR (7)*	*0.005-25.41*	*Yokota et al., 2017*
Marchantiaceae				
Common liverwort (Marchantia polymorpha L.)	*culture cells*	*BR (3)*	*unspecified*	*Park et al., 1999*
	thallus	*CS*	*0.006-0.038*	*Yokota et al., 2017*
	thallus	*BR (6)*	*0.001-0.139*	*Yokota et al., 2017*
	on agar medium	*CS*	*0.007*	*Yokota et al., 2017*
	on agar medium	*BR (5)*	*0.002-0.119*	*Yokota et al., 2017*
Onocleaceae				
Bead fern (Onoclea sensibilis)	*vegetative frond*	*CS*	*0.003*	*Yokota et al., 2017*
	vegetative frond	*BR (3)*	*0.063-0.19*	*Yokota et al., 2017*
Fiddlehead fern (Matteuccia struthiopteris)	*vegetative frond*	*CS*	*0.016*	*Yokota et al., 2017*
	vegetative frond	*BR (3)*	*0.15-1.175*	*Yokota et al., 2017*
Osmundaceae				
Asian royal fern (Osmunda japonica)	*vegetative frond*	*CS*	*0.004-0.005*	*Yokota et al., 2017*
	vegetative frond	*BR (11)*	*0.007-202.9*	*Yokota et al., 2017*
Selaginellaceae				
Spikemoss (Selaginella moellendorffii)	*frond*	*CS*	*0.02*	*Yokota et al., 2017*
	frond	*BR (2)*	*<0.042-0.084*	*Yokota et al., 2017*
Blue Spikemoss (Selaginella uncinata)	*frond*	*CS*	*0.006*	*Yokota et al., 2017*
	frond	*BR (6)*	*0.007-0.275*	*Yokota et al., 2017*

Family/Species	*Examined part*	*Brassino-steroid*[1]	*content µg/kg fr. wt.*[2]	*References*
Thelypteridaceae				
Japanese Beech Fern (Thelypteris decursive-pinnata)	*fertile frond*	*CS*	*0.015*	*Yokota et al., 2017*
	fertile frond	*BR (7)*	*0.025-5.119*	*Yokota et al., 2017*
Marsh fern (Thelypteris palustris)	*vegetative frond*	*BR (6)*	*0.002-1.122*	*Yokota et al., 2017*
Algae				
Chaetophoraceae				
Green algae (Stigeoclonium nanum)	*cultured cells*	*BL*	*168.7 µg/kg dr. wt*	*Stirk et al., 2013*
	cultured cells	*CS*	*144.9 µg/kg dr. wt*	*Stirk et al., 2013*
Chlamydomonadaceae				
Chlamydomonas reinhardtii	*cultured cells*	*BL*	*162.9 µg/kg dr. wt*	*Stirk et al., 2013*
	cultured cells	*CS*	*153.8 µg/kg dr. wt*	*Stirk et al., 2013*
Protococcus viridis	*cultured cells*	*BL*	*211.6 µg/kg dr. wt*	*Stirk et al., 2013*
	cultured cells	*CS*	*134.8 µg/kg dr. wt*	*Stirk et al., 2013*
Chlamydomonadaceae				
	cultured cells	*BL*		*Stirk et al., 2013*
Chlorellaceae				
Green algae (Clorella vulgaris)	*cultured cells*	*BL*	*0.07*	*Bajguz, 2009*
Green algae (Clorella vulgaris)	*cultured cells*	*CS*	*0.47*	*Bajguz, 2009*
Green algae (Clorella vulgaris)	*cultured cells*	*BR (5)*	*0.18-0.39*	*Bajguz, 2009*
Green algae (Clorella pyrenoidosa)	*cultured cells*	*BL*	*253 µg/kg dr. wt*	*Stirk et al., 2013*
	cultured cells	*CS*	*158 µg/kg dr. wt*	*Stirk et al., 2013*
Green algae (Clorella vulgaris)	*cultured cells*	*BL*	*193.3 µg/kg dr. wt*	*Stirk et al., 2013*
	cultured cells	*CS*	*215.3 µg/kg dr. wt*	*Stirk et al., 2013*
Green algae (Clorella minutissima)	*cultured cells*	*BL*	*306.5 µg/kg dr. wt*	*Stirk et al., 2013*
	cultured cells	*CS*	*215.3 µg/kg dr. wt*	*Stirk et al., 2013*
Chlorococcaceae				
Green algae Chlorococcum ellipsoideum	*cultured cells*	*BL*	*168.7 µg/kg dr. wt*	*Stirk et al., 2013*
	cultured cells	*CS*	*105.7 µg/kg dr. wt*	*Stirk et al., 2013*
Green algae Nautococcus mamillatus	*cultured cells*	*BL*	*115.8 µg/kg dr. wt*	*Stirk et al., 2013*
	cultured cells	*CS*	*99.9 µg/kg dr. wt*	*Stirk et al., 2013*
Green algae Spongiochloris excentrica	*cultured cells*	*BL*	*131.2 µg/kg dr. wt*	*Stirk et al., 2013*
	cultured cells	*CS*	*108.5 µg/kg dr. wt*	*Stirk et al., 2013*
Coccomyxaceae				
Green algae Coccomyxa sp.	*cultured cells*	*BL*	*205.8 µg/kg dr. wt*	*Stirk et al., 2013*
	cultured cells	*CS*	*177.1 µg/kg dr. wt*	*Stirk et al., 2013*
Hydrodictyaceae				
Green algae (Hydrodictyon reticulatum (L.) Lager	*cultured cells*	*24-epiCS*	*0.3*	*Yokota et al., 1987b*[3]
	cultured cells	*28-homoCS*	*4*	*Yokota et al., 1987b*[3]
Klebsormidiaceae				
Green algae (Klebsormidium flaccidum)	*cultured cells*	*BL*	*548.7 µg/kg dr. wt*	*Stirk et al., 2013*
		CS	*429.1 µg/kg dr. wt*	*Stirk et al., 2013*

Family/Species	*Examined part*	*Brassino-steroid*[1]	*content µg/kg fr. wt.*[2]	*References*
Neochloridaceae				
Green algae (Poloidion didymos)	*cultured cells*	*BL*	*167.3 µg/kg dr. wt*	*Stirk et al., 2013*
	cultured cells	*CS*	*172.8 µg/kg dr. wt*	*Stirk et al., 2013*
Palmellaceae				
Green algae (Gyoerffyana humicola)	*cultured cells*	*BL*	*270.9 µg/kg dr. wt*	*Stirk et al., 2013*
	cultured cells	*CS*	*201.1 µg/kg dr. wt*	*Stirk et al., 2013*
Prasiolaceae				
Green algae (Stichococcus bacillaris)	*cultured cells*	*BL*	*291.8 µg/kg dr. wt*	*Stirk et al., 2013*
	cultured cells	*CS*	*242.7 µg/kg dr. wt*	*Stirk et al., 2013*
Protosiphonaceae				
Green algae (Protosiphon botryoides)	*cultured cells*	*BL*	*100.6 µg/kg dr. wt*	*Stirk et al., 2013*
	cultured cells	*CS*	*74 µg/kg dr. wt*	*Stirk et al., 2013*
Scenedesmaceae				
Green algae (Acutodesmus acuminatus)	*cultured cells*	*BL*	*125.1 µg/kg dr. wt*	*Stirk et al., 2013*
	cultured cells	*CS*	*105.5 µg/kg dr. wt*	*Stirk et al., 2013*
Green algae (Acutodesmus incrassatulus)	*cultured cells*	*BL*	*124.8 µg/kg dr. wt*	*Stirk et al., 2013*
	cultured cells	*CS*	*92.6 µg/kg dr. wt*	*Stirk et al., 2013*
Green algae (Desmodesmus armatus)	*cultured cells*	*BL*	*125.1 µg/kg dr. wt*	*Stirk et al., 2013*
	cultured cells	*CS*	*109.3 µg/kg dr. wt*	*Stirk et al., 2013*
Green algae (Scotiellopsis terrestris)	*cultured cells*	*BL*	*336.9 µg/kg dr. wt*	*Stirk et al., 2013*
	cultured cells	*CS*	*235.9 µg/kg dr. wt*	*Stirk et al., 2013*
Green algae (Coelastrum microporum)	*cultured cells*	*BL*	*199.2 µg/kg dr. wt*	*Stirk et al., 2013*
	cultured cells	*CS*	*158.3 µg/kg dr. wt*	*Stirk et al., 2013*
Selenastraceae				
Green algae (Monoraphidium contortum)	*cultured cells*	*BL*	*284.9 µg/kg dr. wt*	*Stirk et al., 2013*
	cultured cells	*CS*	*195 µg/kg dr. wt*	*Stirk et al., 2013*
Green algae (Raphidocelis subcapitata)	*cultured cells*	*BL*	*58.6 µg/kg dr. wt*	*Stirk et al., 2013*
	cultured cells	*CS*	*58.7 µg/kg dr. wt*	*Stirk et al., 2013*
Trebouxiaceae				
Green algae (Myrmecia bisecta)	*cultured cells*	*BL*	*202.4 µg/kg dr. wt*	*Stirk et al., 2013*
	cultured cells	*CS*	*164.3 µg/kg dr. wt*	*Stirk et al., 2013*
Ulotrichaceae				
Green algae (Ulothrix sp.)	*cultured cells*	*BL*	*84.9 µg/kg dr. wt*	*Stirk et al., 2013*
	cultured cells	*CS*	*74.2 µg/kg dr. wt*	*Stirk et al., 2013*
Crystoseiraceae				
Brown algae (Cystoseira myrica (Gmelin) Agardh)	*whole plant*	*BR*	*unspecified*	*Hamdy et al., 2009*
Fungi				
Cercospora arachidicola	*unspecified*	*unspecified*	*unspecified*	*Zakharychev, 1999*[3] *in Tsavkelova et al., 2006*
Processed foods				
Juice and Wines				
Apple juice	*juice*	*24-epiBL*	*12*	*Khripach et al., 2013*

Family/Species	*Examined part*	*Brassino-steroid*[1]	*content µg/kg fr. wt.*[2]	*References*
Apple juice	*juice*	*BL*	*1.7*	*Khripach et al., 2013*
Apple juice	*juice*	*28-homoBL*	*3*	*Khripach et al., 2013*
Grape juice	*juice*	*24-epiBL*	*1.7*	*Khripach et al., 2013*
Grape juice	*juice*	*BL*	*1.8*	*Khripach et al., 2013*
Grape juice	*juice*	*28-homoBL*	*0.4*	*Khripach et al., 2013*
Pineapple juice	*juice*	*24-epiBL*	*3*	*Khripach et al., 2013*
Pineapple juice	*juice*	*BL*	*1.6*	*Khripach et al., 2013*
Pineapple juice	*juice*	*28-homoBL*	*0.5*	*Khripach et al., 2013*
Birch juice	*juice*	*24-epiBL*	*0.5*	*Khripach et al., 2013*
Birch juice	*juice*	*BL*	*1.2*	*Khripach et al., 2013*
Birch juice	*juice*	*28-homoBL*	*0.1*	*Khripach et al., 2013*
Dry red wine (Merlot)	*wine*	*24-epiBL*	*3*	*Khripach et al., 2013*
Dry red wine (Merlot)	*wine*	*BL*	*10*	*Khripach et al., 2013*
Dry red wine (Merlot)	*wine*	*28-homoBL*	*4.2*	*Khripach et al., 2013*
Honey				
Honey		*24-epiBL*	*7.4*	*Khripach et al., 2013*
Honey		*BL*	*1*	*Khripach et al., 2013*

[1] *24-epiBL = 24-Epibrassinolide; 24-epiCS=24-Epicastasterone (precursor of 24-Epibrassinolide); BL = Brassinolide; CS=Castasterone (precursor of Brassinolide); 28-homoBL = 28-Homobrassinolide; 28-homoCS = 28-Homocastasterone (precursor of 28-Homobrassinolide); BR (Nr.)= Other Brassinosteroids (Number)*

[2] *Amount of Brassinosteriod is expressed in µg/kg fresh weight, if not specified otherwise*

[3] *Cited in the review publications Bajguz and Tretyn (2003) and Hayat and Ahmad (2011).*

24-Epibrassinolide elicits and activates the plant's self-defence mechanisms mediating the plant's resistance to unfavourable environmental conditions, (e.g. salinity, drought, cold and heat stress) and fungal diseases.

Application of brassinosteroids leads to a complex sequence of biochemical reactions such as activation or suppression of key enzymatic reactions, induction of protein synthesis and the production of various chemical defence compounds (Bajguz and Hayat, 2009). Brassinosteroid treated plants are not only more tolerant to biotic but also to abiotic stresses, providing a solution for problems that could arise in agriculture in the course of the climate change (Eremina et al., 2016).

Humans are constantly exposed to 24-Epibrassinolide through consumption of plants and plant organs, e.g. seeds, roots, and leafs (0.22 - 378 µg/kg), as well as other natural and processed foods such as honey (7.4 µg/kg), fruit juices (0.5 - 12 µg/kg) and wine (3 µg/kg) (Table A-2) and thus 24-Epibrassinolide has no relevant toxicity hazard towards humans.

EFSA (2012) has even concluded that plant sterols (which includes 24-Epibrassinolide) are not only of low risk for the human consumer but necessary for a healthy diet as they are contributing to lowering the LDL-cholesterol levels, which is pivotal for the prevention of coronary heart diseases. Therefore, a daily intake of up to 3 g of plant sterols per day is highly recommended by EFSA (see CA 5.9.2).

Brassinosteroids are also non-toxic to non-target organisms. Mammals, aquatic organisms, insects, and soil organisms are constantly exposed to Brassinosteroids through the consumption of Brassinosteroids contained in higher and lower plants (present in soil, fresh- and seawater). Furthermore, no effects on soil microorganisms are expected. Not only are certain soil microorganisms able to metabolize Brassinosteroids, but some microorganisms are also able to synthesize Brassinosteriods themselves (Tsavkelova et al., 2006).

Non-target soil organisms are constantly exposed to Brassinosteroids, not only from the constant release of Brassinosteroid from decaying plant material (e.g. Aremu et al., 2015) but also from the

Brassinosteroid precursors, campesterol, sitosterol, and stigmasterol. These precursors are known root exudates and are involved in the mediation of interactions in the rhizosphere, which includes the symbiotic associations with beneficial microbes, such as mycorrhizae, rhizobia, and plant growth-promoting rhizobacteria (PGPR) (Badri and Vivanco, 2009).

Due to the constant formation and decomposition of plant root systems, the presence of seeds, pollen, and decomposing plant material and the release of Brassinosteroids from decomposing organic matter (e.g. Aremu et al., 2015) as well as the vast number of other Brassinosteroid producing organisms such as algae in the environment, Brassinosteroids – and other pytosterols – are naturally present in all environmental compartments including soil e.g. Aremu et al., 2015) and water-bodies including sediment (Hassett & Lee, 1977; Mudge et al., 1999).

In addition to that, bioaccumulation is not expected as Brassinosteroids are readily absorbed and metabolised by higher and lower plants (e.g. Nishikawa et al., 1994), diatoms (e.g. Mekhalfi et al., 2012), green algae (e.g. Bajguz, 2011), fungi (e.g. Voigt et al., 1993), mycobacteria (e.g. Vorbrot et al., 1991), and cyanobacteria (e.g. Saygideger and Deniz, 2008). As Brassinosteroids are phylogenetically ancient phytohormones, it can be expected that each organism has developed its own co-evolutionary mechanism to metabolise these phytohormones. It was further found that Brassinosteroid synthesis in plants is naturally triggered for example by microorganisms (Asari et al., 2017).

24-Epibrassinolide can be considered as low risk active substance in accordance with Regulation (EC) 1107/2009, Annex II point 5, as it is not classified as carcinogenic, mutagenic, toxic to reproduction, sensitising, very toxic or toxic, explosive or corrosive and it is not considered persistent, bio-accumulating, endocrine disrupting or neuro- or immunotoxic. Further, it fulfils all low risk criteria indicated in the draft working documents for the purpose of a possible amendment of the current low-risk criteria (Sante/xxxxx/2015 rev. 2, July 2015). In addition, it is a natural, ubiquitous occurring plant molecule, which is expected to have no negative effects on the environment, non-target organism or humans.

24-Epibrassinolide has a very low toxicity profile and is ubiquitous distributed in the plant kingdom (please see Table A-2) and therefore fulfils criterion 3 of SANCO/11188/2013 Rev. 2 of 14 September 2015: "The compound has no identified hazardous properties". In addition, criterion 4 of SANCO/11188/2013 Rev. 2 of 14 September 2015: "Natural exposure is higher than the one linked to the use as PPP", is met. Therefore, inclusion into Annex IV of Regulation (EC) N° 396/2005 is requested, as no maximum residue levels are required.

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