European Commission

Combined Draft (Renewal) Assessment Report prepared according to Regulation (EC) N° 1107/2009
and
Proposal for Harmonised Classification and Labelling (CLH Report) according to Regulation (EC) N° 1272/2008

GIBBERELLINS (GA4, GA7)

Volume 3 – B.9 (AS)

Rapporteur Member State: Slovenia
Co-Rapporteur Member State: Slovakia

Version History

When	What
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B.9. ECOTOXICOLOGY DATA

Introduction

This document has been prepared to evaluate the European Gibberellins Task Force (Valent Biosciences Corporation (Sumitomo Chemical Agro Europe), Fine Agrochemicals Ltd, Globachem NV) application for EU renewal of the Annex I inclusion of active substance gibberellins (GA4, GA7). The document supplements and updates the corresponding Annex B section of the Draft Assessment Report produced during the first review of gibberellins (2005 - 2011).
Gibberelin has been identified as a presumed low-risk active substance in the Commission working document on the AIR-IV renewal programme (SANTE-2016-10616-rev 8). The EU Gibberellin Task Force (EGTF) proposes that Gibberelin is a low risk active substance according to Regulation (EC) 1107/2009 as amended by Commission Regulation 2017/1432.

In this report studies submitted for the first inclusion of gibberellins in Annex I to Directive 91/414/EEC and for the renewal of the approval of gibberellins have been evaluated.

Previous EU assessment

The dossier to support the first inclusion of gibberellins in Annex I to Directive 91/414/EEC was submitted to Hungary as the Rapporteur Member State in June 2005. The Draft Assessment Report is dated July 2006. Final Addendum to Draft Assessment Report, containing all individually submitted addenda on gibberellins, was compiled by EFSA in October 2011.

Structure of this document

Summaries of available data and overall assessments of each sub-section, as well as the exposure assessments, generally are not included in this document. Instead these parts of the assessment are included in Vol. 1, Level 2. The reason behind this structure is to avoid repetition and facilitate revisions of the assessment. As a result, this Annex B only contains the presentation and evaluation of individual study reports on the active substance.

In each section of this document, the following headings (a)-b)) occur:

a) Previous evaluation (2005-2011)
Under this heading study reports submitted for the first inclusion of gibberellins in Annex I to Directive 91/414/EEC are summarised. These studies have been re-evaluated for the purpose of the renewal in the light of current scientific and technical knowledge. The endpoints from the studies were also re-assessed and if considered relevant, re-calculated.

b) Evaluation of additional data for the purpose of renewal of Annex I inclusion
Under this heading studies submitted prior to Annex I inclusion, but no evaluation of such material was presented in the form of Addenda to the DAR and studies that were submitted to support the application for renewal of Annex I inclusion are evaluated, i.e. new studies.

B.9.1. EFFECTS ON BIRDS AND OTHER TERRESTRIAL VERTEBRATES

B.9.1.1. Effects on birds

B.9.1.1.1. Acute oral toxicity to Birds

a) Previous evaluation (2005-2011)

No data are available assessing the acute oral toxicity of gibberellins GA4/GA7 to birds. However, on the basis of the structural and functional similarities between gibberellic acid (GA3) and gibberellins GA4/GA7, an acute oral toxicity study testing the effects of gibberellic acid, GA3, on the Northern Bobwhite (*Colinus virginianus)* was submitted and evaluated as part of the EU review for inclusion of GA4/GA7 in Annex I and is available in the EU DAR. This study was considered acceptable to address the acute avian risk to GA4/GA7 in the EFSA conclusion based on the similarities between GA3 and GA4/GA7 and the high margin of safety obtained with the risk assessment for GA3. This study is considered appropriate for the current assessment to support renewal of GA4/GA7 and no new studies are submitted assessing the acute oral toxicity to birds. Full details of the study are provided in the EU DAR and related documents and references are listed in DRAR Vol.2.

PREVIOUS EVALUATION	This study was evaluated in the original DAR and has been considered by EFSA. No new evaluation has been performed. The conclusion has not been changed.
Data point addressed:	CA 8.1.1.1/01 (II A 8.1.1/01 in original DAR)
Author(s) (year):	[redacted] (1996)
Title:	Falgro Technical: An acute oral toxicity study with the Northern Bobwhite
Laboratory report / project number:	[redacted]
Testing facility:	[redacted]
Published:	No
Test guideline used:	EPA FIFRA 71-1
Deviations:	None
GLP:	Yes
EU Agreed Endpoint:	LD_{50} > 2250 mg/kg bw

Executive summary

The short-term dietary toxicity of gibberellic acid (GA3) was determined in northern bobwhite quail (*Colinus virginianus*). No treatment-related mortalities occurred. The short-term dietary LC_{50} is > 5200 ppm, the highest dietary concentration tested, equivalent to a daily dose of > 904 mg a.s./kg bw/d. In the absence of treatment-related effects on any observed parameters, the NOEC is considered to be 5200 mg a.s./kg diet.

I MATERIALS AND METHODS

MATERIALS

Test material:	Gibberellic acid (GA3)
Lot/Batch No.:	109/95
Purity:	91.1% (w/w)
Stability of test compound:	Not reported, but may be considered to be adequately stable under the conditions of this study based on the behaviour observed in other tests.

Test organism:	Northern bobwhite quail (*Colinus virginianus*)
Source:	[redacted]
Age at test initiation:	25 weeks, with bodyweights ranging from 208 to 273 g.
Dosage levels:	Six treatment groups: 0 (vehicle control), 292, 486, 810, 1350 and 2250 mg/kg bw. A single dose was administered in corn oil by oral intubation.
Number of birds/group	Five male and five female birds/treatment group.
Duration:	14 days after dose administration.
Conditions:	Birds were housed by sex in groups of five according to treatment in floor pens measuring 78 × 51 cm. Pens were constructed from galvanised steel with a wire mesh floor. Throughout acclimation and testing all test birds were fed a diet formulated to test facility specifications, containing no antibiotics or growth promoters. Water from the public mains supply and feed were provided *ad libitum* during acclimation (15 days) and the test, but food was withdrawn for an overnight starvation period of *ca*. 15 h prior to dosing.
Temperature:	Mean 21.9°C ± 0.7°C SD.
Relative humidity:	Mean 25% ± 18% SD.
Observations:	Birds were observed daily during the study and at frequent intervals during the post-treatment period. Mortalities, bird health and clinical signs were recorded at each observation.
Statistical analysis:	Not required.

II RESULTS AND DISCUSSION

A FINDINGS

There were no mortalities in the control group or any of the groups dosed with the test substance. All control birds were normal in appearance and behaviour and there were no overt or clinical signs of toxicity in any bird following dosing.

There was no evidence of any treatment-related effect on food consumption or body weight.

B VALIDITY

The test was considered valid.

III CONCLUSIONS

The acute oral toxicity of gibberellic acid (GA3) was determined in northern bobwhite quail (*Colinus virginianus*) following administration of a single dose by intubation. No mortalities occurred. The acute oral LD_{50} is > 2250 mg a.s./kg bw, the highest dose tested. In the absence of treatment-related effects on any observed parameters, the NOEL is considered to be 2000 mg a.s./kg bw.

RMS comments and conclusions:

This study was conducted in compliance with an older guideline EPA FIFRA 71-1. This study is being evaluated against test guideline US EPA OCSPP 850.2100 Avian Acute Oral Toxicity Test, which was developed in 2012 based on EPA FIFRA 71-1.

The US EPA OCSPP 850.2100 Avian Acute Oral Toxicity Test guideline is designed to develop data, specifically both a median lethal dose (LD_{50}) and slope of the dose-response relationship, for acute oral toxicity to upland game birds (e.g., northern bobwhite (*Colinus virginianus*)), water fowl (e.g., mallard duck (*Anas platyrhynchos*)), or passerine species (e.g., house sparrow (*Passer domesticus*), zebra finch (*Taeniopygia guttata*), red-wing blackbird (*Agelaius phoeniceus*)) of chemical substances and mixtures (" test chemicals" or " test substances") subject to environmental effects test regulations.

Validity: According to US EPA OCSPP 850.2100 test guideline this test would be considered unacceptable or invalid if one or more of the following conditions occurred:

- Birds were not randomly assigned to treatment and control pens **(in test: condition fulfilled)**
- More than 10% of the control birds died during the test **(in test: control mortality = 0%, condition fulfilled)**
- A minimum of ten birds were not used for each dose level of the test substance and control **(in test: 10 birds per treatment, condition fulfilled)**
- The test substance was not orally administered, via either capsule or gavage **(in test: birds were given single oral dose by intubation, condition fulfilled)**
- In the definitive test a minimum of five dose levels of the test substance, plus an appropriate control, were not tested **(in test: control and five concentrations of GA3, condition fulfilled)**

There were deviations from Good Laboratory Practice standards in this study: Verification of the test concentration, stability and homogeneity of the test substance in the diluent were not determined. The stability of the test substance under the conditions of storage at the test site was not determined in accordance with GLP standards.

Photoperiod in the study was 8h of light per day, which is less than 10h, as required by US EPA OCSPP 850.2100 test guideline. No justification is given for this deviation. However, the photoperiod is in line with OECD guideline 223 Avian Acute Oral Toxicity test. The RMS considers this to be a minor deviation that does not affect the validity of the result.

The humidity during the test was 25% ± 18% SD, which is less than the recommended relative humidity between 45 and 70%. The US EPA OCSPP 850.2100 test guideline states that humidity is not as critical as other variables. OECD guideline 223 Avian Acute Oral Toxicity test does not define the required humidity range, however, it states that conditions should be stable. The RMS considers this to be a minor deviation that does not affect the result.

The NOEL is 2250 mg/kg bw.

Acceptability of the analytical methods used in the test: Not applicable.

Endpoints: The endpoint relevant for risk assessment is LD_{50} > 2250 mg/kg bw.

Conclusion of the RMS: The test is considered valid.

RMS comments and conclusions:

For animal welfare reasons the read across approach is used for risk assessment of gibberellins GA4/GA7 in birds. Toxicity data on gibberellic acid GA3 is considered acceptable for risk assessment of gibberellins GA4/GA7 based on the similarities between GA3 and GA4/GA7, the high margin of safety obtained with risk assessment for GA3 and the observed biological transformation of GA4/GA7 to GA3. Therefore, in current re-registration an acute toxicity study with GA3 is submitted by the applicant for risk assessment of GA4/GA7 in birds.

However, two acute avian oral toxicity studies with GA3 were evaluated as part of the EU review for the first inclusion of GA4/GA7 in Annex I and are available in the EU DAR for GA4/GA7 (2011). The applicant submitted only one of these studies for re-registration. For the sake of completeness, the original summary of the other study is presented below. This study has been evaluated in the original DAR and has been considered by EFSA. It was found to be valid. The study gives an endpoint LD_{50}> 2250 mg/kg bw. This is the same as the endpoint in the study that has been submitted for re-registration.

Study 2 (II A 8.1.1/02) Northern Bobwhite

Bird acute toxicity with Gibberellic acid (GA_3) has been already studied and reported in the literature. Conclusions from the literature are given herein:

Reference:	**US-EPA (1995)**. Reregistration Eligibility Decision (R.E.D), Gibberellic acid. Report N°: [redacted]: [redacted] (1991) Gibberellic Acid Technical Material Code 33690 (Encapsulated): An acute oral toxicity study with the Northern Bobwhite. Ltd project No.: 161-121. Unpublished study prepared by [redacted]. Master record identification number of [redacted]
Testing laboratory:	[redacted]
Guideline(s):	US-EPA. Pesticide Assessment Guidelines, Series 154B-6.
Validity:	Study is considered to be valid and is accepted.
Material and methods:	
Test substance:	Gibberellic acid (GA_3).
Test organism:	Northern Bobwhite (*Colinus virginianus).*
Duration:	Birds were given a single oral dose, by intubation and were observed for 14 days following dosing.
Conclusions	
LD_{50}:	The acute oral LD_{50} value for Gibberellic Acid Technical Material Code 33690 to Northern bobwhite is in excess of 2250 mg/kg bw.
NOEL:	There were no treatment related effects on any of the measured parameters. Therefore, 2250 mg/kg bw is considered to be the no-effect level.

RMS comments and conclusions:

Three acute avian oral toxicity studies with GA3 were evaluated as part of the EU review for the inclusion of GA3 in Annex I and are available in the EU DAR for GA3. Two of these [redacted] 1996 and [redacted] 1991) give an endpoint LD_{50}>2250 mg as/kg bw. The thirds study ([redacted] 1991) gives an endpoint LD_{50}>2000 mg as/kg bw. This endpoint (LD_{50}>2000 mg as/kg bw) is worst case and was used in the risk assessment in the original DAR for GA3 and is also stated in EFSA peer review (EFSA Journal 2012;10(1):2507). The study by Hakin has also been submitted now for re-registration of GA3 and the endpoint LD_{50}>2000 mg as/kg bw is being used for risk assessment in re-registration of GA3.

No acute toxicity studies with GA4/GA7 were available at first inclusion of GA4/GA7 on Annex I. For animal welfare reasons no new studies with GA4/GA7 were requested and the read across approach was used for risk assessment of GA4/GA7 in birds. Toxicity data on gibberellic acid GA3 was considered acceptable for risk assessment of gibberellins GA4/GA7. However only two of the three acute avian oral toxicity studies with GA3 were evaluated as part of EU review for the inclusion of GA4/GA7 in Annex I. These are studies by [redacted] and Campbell that give the endpoint LD_{50}> 2250 mg as/kg bw. The third study by [redacted] 1991 that gives an endpoint LD_{50}>2000 mg as/kg bw and represents the worst-case endpoint of all studies for acute toxicity to birds was not evaluated for first inclusion of GA4/GA7 on Annex I. The endpoint LD_{50}> 2250 mg as/kg bw was used for acute risk assessment of GA4/GA7 in birds in original DAR and is also stated in EFSA peer review of GA4/GA7 (EFSA Journal 2012;10(1):2502).

It is not clear to the RMS why the study by [redacted] (1991) was not submitted for the first inclusion of GA4/GA7 to Annex I and why it is not submitted now for re-registration of GA4/GA7. The RMS is of the opinion that if read across from GA3 is used for risk assessment of GA4/GA7 the worst-case endpoint from GA3 (LD_{50}>2000 mg as/kg bw) should be used for risk assessment of GA4/GA7.

Commission regulation (EU) No 283/2013 requires that all available biological data and information which is relevant to the assessment of the ecotoxicological profile of the active substance be reported (Introduction to Section 8 – Ecotoxicological studies). To meet this requirement the RMS will include in this dossier and in the risk assessment the study by [redacted], as it was submitted for re-registration of gibberellic acid (GA3). The study is summarised and evaluated below. The acute toxicity endpoint to be used in risk assessment of GA4/GA7 in birds is LD_{50}>2000 mg as/kg bw.

PREVIOUS EVALUATION	This study was evaluated in the original DAR and has been considered by EFSA. No new evaluation has been performed. The conclusion has not been changed.
Data point addressed:	CA 8.1.1.1/03 (II A 8.1.1/02 in original DAR)
Author(s) (year):	[redacted] (1991a)
Title:	Gibberellic Acid Acute Oral Toxicity (LD_{50}) to Mallard Duck
Laboratory report / project number:	[redacted]
Testing facility:	[redacted]
Published:	No
Test guideline used:	EPA FIFRA 71-1
Deviations:	None
GLP:	Yes

EU Agreed Endpoint:	LD_{50} > 2000 mg/kg bw

Executive summary

The acute oral toxicity of gibberellic acid (GA3) was determined in mallard duck (*Anas platyrhynchos*) following administration of a single dose by intubation. No mortalities occurred. The acute oral LD_{50} is > 2000 mg a.s./kg bw, the highest dose tested. In the absence of treatment-related effects on any observed parameters, the NOEL is considered to be 2000 mg a.s./kg bw.

I MATERIALS AND METHODS

MATERIALS

Test material:	Gibberellic acid
Lot/Batch No.:	BIGA/00/
Purity:	91.2% (w/w)
Stability of test compound:	Adequately stable under the conditions of the study. Shown to be stable in corn oil formulations at inclusion rates of 10% and 40% (w/v) over 4 hours.

Test organism:	Mallard duck (*Anas platyrhynchos*)
Source:	[redacted]
Age at test initiation:	11 months, with bodyweights ranging from 920 to 1195 g.
Dosage levels:	Four treatment groups: 0 (vehicle control), 500, 1000 and 2000 mg/kg bw. A single dose was administered in corn oil by oral intubation.
Number of birds/group	Five male and five female birds/treatment group.
Duration:	14 days after dose administration.
Conditions:	Birds were housed by sex in groups of five according to treatment in floor pens measuring 1.84 × 1.20 m. Pens were constructed from galvanised steel with a wire mesh floor and were fitted with an automatic drinker and food hopper. Throughout acclimation and testing all test birds were fed a pelleted layer diet, containing no antibiotics or growth promoters. Water from the public mains supply and feed were provided *ad libitum* during acclimation (15 days) and the test, but food was withdrawn for an overnight starvation period of *ca*. 19 h prior to dosing.
Temperature:	Mean min and max 15 and 19°C, respectively.
Relative humidity:	Mean 93%.
Observations:	Birds were observed daily during the study and at frequent intervals during the post-treatment period. Mortalities, bird health and clinical signs were recorded at each observation.
Statistical analysis:	Not required.

II RESULTS AND DISCUSSION

A FINDINGS

A single bird in group 2 was found dead on Day -1 with feathers missing from its head, neck and back and was replaced by a spare bird. There were no mortalities following dosing. No clinical signs of toxicity were observed in any bird.

There was no evidence of any treatment-related effect on food consumption or body weight.

B VALIDITY

The test was considered valid.

III CONCLUSIONS

The acute oral toxicity of gibberellic acid (GA3) was determined in mallard duck (*Anas platyrhynchos*) following administration of a single dose by intubation. No mortalities occurred. The acute oral LD_{50} is > 2000 mg a.s./kg bw, the highest dose tested. In the absence of treatment-related effects on any observed parameters, the NOEL is considered to be 2000 mg a.s./kg bw.

RMS comments and conclusion:

This study was conducted in compliance with an older guideline EPA FIFRA 71-1. This study is being evaluated against test guideline US EPA OCSPP 850.2100 Avian Acute Oral Toxicity Test, which was developed in 2012 based on EPA FIFRA 71-1.

The US EPA OCSPP 850.2100 Avian Acute Oral Toxicity Test guideline is designed to develop data, specifically both a median lethal dose (LD_{50}) and slope of the dose-response relationship, for acute oral toxicity to upland game birds (e.g., northern bobwhite (*Colinus virginianus*)), water fowl (e.g., mallard duck (*Anas platyrhynchos*)), or passerine species (e.g., house sparrow (*Passer domesticus*), zebra finch (*Taeniopygia guttata*), red-wing blackbird (*Agelaius phoeniceus*)) of chemical substances and mixtures (" test chemicals" or " test substances") subject to environmental effects test regulations.

Validity: According to US EPA OCSPP 850.2100 test guideline this test would be considered unacceptable or invalid if one or more of the following conditions occurred:

- Birds were not randomly assigned to treatment and control pens **(in test: condition fulfilled)**
- More than 10% of the control birds died during the test **(in test: control mortality = 0%, condition fulfilled)**
- A minimum of ten birds were not used for each dose level of the test substance and control **(in test: 10 birds per treatment, condition fulfilled)**
- The test substance was not orally administered, via either capsule or gavage **(in test: birds were given single oral dose by intubation, condition fulfilled)**
- In the definitive test a minimum of five dose levels of the test substance, plus an appropriate control, were not tested **(in test: control and three concentrations of GA3, condition NOT fulfilled)**

The guideline suggests that for more consistent responses the range of body weights should be no greater than ±10% of the mean body weight for the test population. The study report does not state mean body weight of test organisms, but rather the range (920 - 1195 g). The RMS considers this to be acceptable.

The study report reports the photo-period 10 hours of continuous light and 14 hours of dark, as required by guidance document.

The mean daily relative humidity during the study was 93%, higher than the recommended 45 – 70%. The RMS considers this to be minor deviation that does not affect the result.

The LD_{10} and LD_{20} values were not reported as this was not a data requirement at the time when study was conducted. The LD_{10} and LD_{20} values could not be determined due to the lack of mortality during the study. For animal welfare reasons, no new study is considered necessary.

GA3 is stable under experimental conditions with mean measure concentrations in the range 108.3% - 114%

of nominal values.

Acceptability of the analytical methods used in the test:

Endpoints: The endpoint relevant for risk assessment is LD_{50} > 2000 mg/kg bw.

Conclusion of the RMS: The test is considered valid.

b) Evaluation of additional data for the purpose of renewal of Annex I inclusion

No additional data was submitted or required for the purpose of renewal of approval of gibberellins GA4/GA7.

B.9.1.1.2. Short-term dietary toxicity to birds

a) Previous evaluation (2005-2011)

No data are available assessing the short-term dietary toxicity of gibberellins GA4/GA7 to birds. However, on the basis of the similarities between gibberellic acid (GA3) and GA4/GA7, a short-term dietary toxicity study testing the effects of gibberellic acid, GA3, on the Northern Bobwhite (*Colinus virginianus)* was submitted and evaluated as part of the EU review for inclusion of GA4/GA7 in Annex I and is available in the EU DAR. This study was considered acceptable to address the avian risk to GA4/GA7 in the EFSA conclusion based on the similarities between GA3 and GA4/GA7 and the high margin of safety obtained with the risk assessment for GA3. The short-term avian risk assessment is now obsolete according to current EFSA guidance (EFSA Journal 2009; 7(12):1438; updated 2010). However, the available study is considered relevant to support renewal of GA4/GA7 as evidence to waive a sub-chronic and reproductive toxicity study (see B.9.1.1.3). No new studies are submitted assessing the short-term dietary toxicity to birds. Full details of the study are provided in the EU DAR and related documents and references are listed in DRAR Vol.2.

PREVIOUS EVALUATION	This study was evaluated in the original DAR and has been considered by EFSA. No new evaluation has been performed. The conclusion has not been changed.
Data point addressed:	CA 8.1.1.2/01 (II A 8.1.2/01 in original DAR)
Author(s) (year):	[redacted] (1996)
Title:	Falgro Technical: A dietary LC_{50} study with the Northern Bobwhite
Laboratory report / project number:	[redacted]
Testing facility:	[redacted]
Published:	No
Test guideline used:	EPA FIFRA 71-2
Deviations:	None
GLP:	Yes
EU Agreed Endpoint:	LC_{50} > 5620 mg/kg feed, equivalent to LD_{50} > 1376 mg/kg bw/d NOEC = 5620 mg/kg feed, equivalent to NOEL = 1376 mg/kg bw/d

Executive summary

The short-term dietary toxicity of gibberellic acid (GA3) was determined in northern bobwhite quail (*Colinus virginianus*). No treatment-related mortalities occurred. The short-term dietary LC_{50} is > 5620 ppm, the highest dietary concentration tested, equivalent to a daily dose of > 1376 mg a.s./kg bw/d. In the absence of treatment-related effects on any observed parameters, the NOEC is considered to be 5620 mg a.s./kg diet.

I MATERIALS AND METHODS

MATERIALS

MATERIALS AND METHODS	
Test material:	Gibberellic acid (GA3)
Lot/Batch No.:	109/95
Purity:	91.1% (w/w)
Stability of test compound:	Adequately stable under the conditions of the study. Mean analytical recovery in diets ranged from 97% to 114% of measured Day 0 concentrations after 5-day storage under test conditions.

Test organism:	Northern bobwhite quail (*Colinus virginianus*)
Source:	Hatched at the test facility.
Age at test initiation:	10 days.
Dosage levels:	Nine treatment groups: four controls and five gibberellic acid at nominal concentrations of 562, 1000, 1780, 3160 and 5620 mg/kg.
Number of birds/group	Ten birds/treatment group.
Duration:	Test or control diets were offered ad libitum during the 5-day treatment period. Test diets were then replaced with basal diet and the birds observed for a further 3 days.
Conditions:	Birds housed according to treatment in batteries of brooding pens, each measuring *ca*. 72 × 90 × 23 cm constructed of galvanised steel wire and sheeting, with 10 birds/pen. Throughout acclimation and testing all test birds were fed a game bird ration formulated to test facility specifications, containing no antibiotic medication. Water from the public mains supply and feed were provided *ad libitum* during acclimation (10 days) and the test.
Temperature:	Mean 24.5°C ± 2.3°C SD.
Relative humidity:	Mean 24% ± 4% SD.
Diet analysis:	Samples of diets were taken for confirmation of inclusion levels. Samples were also taken from the 562 and 5620 ppm (nominal) diets on the day of preparation to confirm homogeneity. Stability samples were taken from all diets at the end of the exposure period (Day 5). All samples were analysed for gibberellic acid by HPLC/UV.
Observations:	Birds were observed at least twice daily during the study and at frequent intervals during the post-treatment period. Mortalities, bird health and clinical signs were recorded at each observation. Group mean bodyweights were determined on days -5, 0 (immediately prior to the introduction of test diets), 5 and 8. Group mean food consumption was measured over days -5 to 1, 1 to 5 (daily) and 6 to 8.
Statistical analysis:	Not required.

II RESULTS AND DISCUSSION

A FINDINGS

Food consumption and bodyweight changes were similar in all groups throughout the study and there was no evidence of any treatment-related effect on these parameters.

There were no mortalities and no clinical signs of toxicity were observed in any bird.

Mean measured gibberellic acid (GA3) concentrations in samples of freshly prepared diets ranged from 92% to 97% of nominals and mean analytical recovery in diets ranged from 97% to 114% of measured Day 0 concentrations after 5-day storage under test conditions. Samples from the 562 and 5620 ppm diets showed that the test substance was homogeneously mixed in the test diets.

Daily dose (mg/kg bw/d) = concentration in food (mg/kg) multiplied by daily food consumption (g per bird per day) divided by body weight (g). At the 5620 ppm dose rate the mean food consumption during the 5-day exposure period was 6 g/bird/day and the mean body weight was 24.5 g (mean of day 0 and day 5 weights). Therefore, the LC_{50} for gibberellic acid exceeds a daily dose of 1376 mg a.s./kg bw/d.

B VALIDITY

The test was considered valid.

III CONCLUSIONS

The short-term dietary toxicity of gibberellic acid (GA3) was determined in northern bobwhite quail (*Colinus virginianus*). No treatment-related mortalities occurred. The short-term dietary LC_{50} is > 5620 ppm, the highest dietary concentration tested, equivalent to a daily dose of > 1376 mg a.s./kg bw/d. In the absence of treatment-related effects on any observed parameters, the NOEC is considered to be 5620 mg a.s./kg diet.

RMS comments and conclusions:

This study was conducted in compliance with an older guideline EPA FIFRA 71-2. The RMS has evaluated this study according to test guideline US EPA OCSPP 850.2200 Avian Dietary Toxicity Test, which was published in 2012 and was developed based on EPA FIFRA 71-2.

The US EPA OCSPP 850.2200 Avian Dietary Toxicity Test guideline is intended for use in developing data, specifically both a median lethal concentration (LC_{50}) and slope of the concentration-response, on the dietary toxicity to young northern bobwhite (*Colinus virginianus*) and mallard (*Anas platyrhynchos*) of chemical substances and mixtures ("test chemicals" or "test substances") subject to environmental effects test regulations.

Validity: According to US EPA OCSPP 850.2200 Avian Dietary Toxicity Test guideline this test would be considered to be unacceptable or invalid if one or more of the following conditions occurred:

- Birds were not randomly assigned to treatment and control pens **(in test: condition fulfilled)**
- More than 10% of the control birds died or became moribund during the test **(in test: no mortalities in control, condition fulfilled)**
- Concentrations of the test substance were not satisfactorily maintained in the diet (levels should be at least 80% of the nominal concentration) throughout the exposure period (i.e., the first 5 days) **(in test: mean measured results were within the range 97% to 114% of nominal, condition fulfilled)**
- Birds were not administered the test substance in their daily diet for 5 consecutive days **(in test: test diets were offered *at libitum* during 5-day treatment period, condition fulfilled)**
- A minimum of 10 young birds were not used for each dietary concentration of the test substance **(in test: eight groups each of 10 birds were allocated to treatment, condition fulfilled)**
- The test substance was not administered in the diet **(in test: test diets were offered *at libitum*, condition fulfilled)**
- In the definitive test a minimum of five dietary levels of the test substance, plus appropriate controls, were not tested **(in test: 5 nominal concentrations and 4 controls, condition fulfilled)**

The photoperiod in this study was 16h of light per day, which is more than the recommended 14h. The photoperiod is in line with the OECD guideline 205 Avian Dietary toxicity test. The RMS considers this to be a

minor deviation that does not affect the validity of the result.

The mean humidity in the study was 24% ± 4% SD, which is outside of the recommended range between 45 and 70%. The test guideline states that relative humidity is not as critical as some other variables. The RMS considers this to be a minor deviation that does not affect the validity of the result.

There was a deviation from Good Laboratory Practice standards in this study: The stability of the test substance under the conditions of storage at the test site was not determined in accordance with GLP standards.

Acceptability of the analytical methods used in the test: /

Endpoints: The endpoint relevant for risk assessment is LC_{50} >1376 mg a.s./kg bw/d.

Conclusion of the RMS: The study is valid.

b) Evaluation of additional data for the purpose of renewal of Annex I inclusion

No additional data was submitted or required for the purpose of renewal of approval of gibberellins GA4/GA7.

B.9.1.1.3. Sub-chronic toxicity and reproduction to birds

a) Previous evaluation (2005-2011)

No studies on the reproductive toxicity of gibberellins GA4/GA7 to birds are available. Acute and short-term dietary toxicity studies to birds have shown that GA3 is of low toxicity to birds. The applicant submitted studies showing acute and short term toxicity to be > 2250 mg/kg bw and > 1376 mg/kg bw/d, respectively. However a study is available that gives an acute toxicity endpoint > 2000 mg/kg bw. As this is worst-case, the RMS will use this value for risk assessment. Exposure of birds from the proposed uses of GA4/GA7 is not expected to exceed exposure from environmental sources of GA4/GA7 including naturally occurring GAs in plant tissues, such as apple seeds and other fruits, leaves and shoots (see CA 8.9/01). As a protective worst-case, the long-term NOEL for birds has been assumed to be 100-fold lower than the NOEL for short-term toxicity in order to assess the long-term risk DRAR Vol.3 CP Novagib B.9). Given that the NOEL for short-term toxicity was found to equate to the highest dose rate tested, this is considered to be an acceptable approach. It is noted that mammalian toxicity data showed GA4/GA7 to have very low chronic toxicity (rat NOAEL in two-generation study = 300 mg/kg bw/d). Further data on sub-chronic toxicity and reproductive toxicity to birds is therefore not considered necessary.

b) Evaluation of additional data for the purpose of renewal of Annex I inclusion

No additional data was submitted or required for the purpose of renewal of approval of gibberellins GA4/GA7.

B.9.1.2. Effects on terrestrial vertebrates other than birds

B.9.1.2.1. Acute oral toxicity to mammals

a) Previous evaluation (2005-2011)

An acute mammalian oral toxicity study with gibberellins GA4/GA7 was evaluated as part of the EU review for the inclusion of GA4/GA7 in Annex I and is available in section B.6 (Toxicology and Metabolism) in the EU DAR. The study was identified in the EFSA conclusion as the critical study for the ecotoxicological risk assessment of mammals, with an LD_{50} in rats > 5000 mg/kg bw. This study is considered appropriate for the current assessment to support renewal of GA4/GA7 and no new studies are submitted assessing the acute oral

toxicity to mammals. Full details of the study are provided in the EU DAR (B.6.2.1.1) and related documents and discussed further in DRAR Vol.3 CA B.6 (CA 5.2.1/02).

b) Evaluation of additional data for the purpose of renewal of Annex I inclusion

No additional data was submitted or required for the purpose of renewal of approval of gibberellins GA4/GA7.

B.9.1.2.2. Long-term and reproduction toxicity to mammals

a) Previous evaluation (2005-2011)

A dietary two-generation reproductive toxicity study in rat with gibberellins GA4/GA7 was evaluated as part of the EU review for the inclusion of GA4/GA7 in Annex I and is available in section B.6 (Toxicology and Metabolism) in the EU DAR. The study was identified in the EFSA conclusion as the critical study for the ecotoxicological risk assessment of mammals, with a NOAEL in rats of 300 mg/kg bw/d. This study is considered appropriate for the current assessment to support renewal of GA4/GA7 and no new studies are submitted assessing the long-term and reproductive toxicity to mammals. Full details of the study are provided in the EU DAR (B.6.6.1) and related documents and discussed further in DRAR Vol.3 CA B.6 (CA 5.6.1/01).

b) Evaluation of additional data for the purpose of renewal of Annex I inclusion

No additional data was submitted or required for the purpose of renewal of approval of gibberellins GA4/GA7.

B.9.1.3. Active substance bioconcentration in prey of birds and mammals

The log P_{ow} values of gibberellins GA4 and GA7 are 2.34 and 2.25, respectively, as agreed by EFSA (EFSA Journal 2012;10(1):2502). Given that the log P_{ow} is lower than the trigger of 3 it is considered that there is no potential for bioaccumulation of GA4/GA7 in fatty tissues and therefore there is no potential for secondary poisoning. Furthermore, GA4, GA7 and other gibberellins are naturally occurring (see CA 8.9/01, and the review of public domain literature on the presence of endogenous gibberellins in higher plants, algae, mosses and lichens as well as the biosynthesis of gibberellins by soil bacteria and fungi included at Vol.3 CA B.8). There is no evidence of bioconcentration in prey of birds and mammals from natural sources of gibberellins.

B.9.1.4. Other data on effects on terrestrial vertebrate wildlife (birds, mammals, reptiles and amphibians)

According to Commission Regulation (EU) No 283/2013 and 284/2013, the risk to amphibians and reptiles shall be addressed. However, in the EU there are no guidance documents issued by the European Food Safety Authority (EFSA) on how to conduct risk assessments for amphibians and reptiles. Nevertheless, there are some specific recommendations in an EU regulatory document (EFSA/2013/3290, aquatic guidance), which are taken here into consideration to address the requirements under point 8.1.4.

The aquatic guidance document (EFSA, 2013) states: "Even if the revised data requirements (Commission Regulation (EU) 283/2013) do not request toxicity tests for amphibian species, amphibians should be included in the aquatic and terrestrial RA of PPPs. Assessment of the risk to amphibians should be based on any existing relevant information. Available relevant data, including data from the open literature, for the substance under consideration should be presented and taken into account in the RA…". Therefore, the availability of studies on the toxicity of the active substances should be considered.

In the case of gibberellins GA4/GA7, there are no studies available, neither in the literature nor in unpublished reports submitted by the applicant, on the toxicity of this active substance to amphibians or reptiles. Therefore, due to the lack of a standard risk assessment and lack of data on the toxicity of the active substance to amphibians and reptiles, a regulatory risk assessment for these organisms is not applicable at this time.

B.9.1.5. Potential for endocrine disruption

The EFSA/ECHA guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009 (EFSA Journal 2018;16(6):5311) foresees a gradual approach to identification of endocrine disrupting properties of active substances. It is recommended to strive for a conclusion on the ED properties with regard to humans and in parallel, using the same database, to strive for a conclusion on mammals as non-target organisms. If the substance under investigation is found to be ED for humans, the assessment need not continue. This is because it is sufficient that the substance meets the ED criteria in one taxonomic group in order to conclude that a substance meets the ED criteria for all non-target organisms. If the substance under investigation is not ED for humans, the population relevance of the observed adverse effects needs to be assessed in order to conclude on ED properties with regard to mammals as non-target organisms. In order to conclude on the ED properties of gibberellins GA4/GA7 with regard to humans new data need to be generated (see Vol 3 CA Section B.6.8.3, toxicological assessment). Until a conclusion is made regarding humans the assessment of the ED properties of gibberellins GA4/GA7 with regard to mammals as non-target organisms cannot proceed.

Where the evidence available indicates that the ED criteria are not met for mammals as non-target organisms, the assessment for non-target organisms should proceed by considering fish and amphibians, because these are the taxa where standardised test methods and knowledge on how to interpret the results are available. Information on other taxa (e.g. birds and reptiles) should be considered if available. In the case of gibberellins GA4/GA7 no relevant information for birds and reptiles is available, therefore no assessment of endocrine disrupting properties in regard to these species was performed.

Please refer to Section B.9.2.4 for detailed assessment of endocrine disrupting properties of gibberellins GA4/GA7 regarding fish and amphibians.

B.9.2. EFFECT ON AQUATIC ORGANISMS

B.9.2.1. Acute toxicity to fish

a) Previous evaluation (2005-2011)

Three acute toxicity studies testing both gibberellins GA4/GA7 (one study) and gibberellic acid GA3 (two studies) to fish were evaluated as part of the EU review for the inclusion of GA4/GA7 in Annex I and are available in the EU DAR. These studies were considered acceptable in the EFSA conclusion for the risk assessment of fish. The critical study testing GA4/GA7 with the Rainbow trout (*Oncorhynchus mykiss*) is considered appropriate for the current assessment to support renewal of GA4/GA7 and no new studies are submitted assessing the acute toxicity to fish. Full details of the study are provided in the EU DAR and related documents and references are listed in DRAR Vol.3 B.9.11. As stated in the residue definition in DRAR Vol 3 CA B.8.6, there are no surface water metabolites of environmental concern and the data provided are therefore limited to the active substance.

PREVIOUS EVALUATION	This study was evaluated in the original DAR and has been considered by EFSA. No new evaluation has been performed. The conclusion has not been changed.
Data point addressed:	CA 8.2.1/01 (II A 8.2.1/01 in original DAR)
Author(s) (year):	[redacted] (2004a)
Title:	Gibberellins A_4 & A_7 – Acute toxicity to rainbow trout (*Oncorhynchus mykiss*) under static-renewal conditions
Laboratory report / project number:	[redacted]
Testing facility:	[redacted]
Published:	No
Test guideline used:	OECD 203 (1992); US EPA OPPTS Draft Guideline 850.1075
Deviations:	None
GLP:	Yes
EU Agreed Endpoint:	96 h (semi-static) LC_{50} >100 mg a.s./L (nominal)

Executive summary

The acute toxicity of gibberellins (GA4/GA7) to rainbow trout (*Oncorhynchus mykiss*) was determined in a 96-hour test under semi-static conditions. No treatment-related mortalities occurred. The 96-hour LC_{50} is > 96 mg a.s./L (mean measured), the highest concentration tested. In the absence of treatment-related mortality or behavioural abnormalities, the NOEC is considered to be 96 mg a.s./L.

I MATERIALS AND METHODS

MATERIALS

Test material:	Gibberellins A4 + A7 (GA4/A7)
Lot/Batch No.:	107-554-CD
Purity:	90.3%
Stability of test compound:	Adequately stable under the conditions of the study. Samples of test media collected during the test performed under semi-static conditions had mean measured concentrations that ranged from 90% to 100% of nominal concentrations.

Test organism:	Rainbow trout (*Oncorhynchus mykiss* Walbaum)
Source:	[redacted]
Age at test initiation:	Juvenile, mean total length 50 mm, mean wet weight 1.2 g.
Treatments:	Six groups: dilution water control and gibberellin treatments at nominal concentrations of 5.7, 12, 25, 47 and 96 mg/L.
Number of fish/group	10/treatment group, with 5 fish per replicate glass aquarium containing 15 L medium.
Duration:	96 hours.
Conditions:	The test was performed under semi-static conditions, with media renewal at 48 h. Measurements of temperature, pH and dissolved oxygen concentrations were made at initiation and termination, and at daily intervals in all vessels. The fish were not fed throughout the exposure period or during the 48 hours that immediately preceded it.
Temperature:	13 to 16°C.
pH:	5.9 to 7.0.
Dissolved oxygen:	6.2 to 9.7 mg/L (>60% ASV).
Total hardness:	44 to 48 mg $CaCO_3$/L.
Photoperiod:	16 hours.
Confirmatory analysis:	Samples were collected from each test vessel at the beginning and the end of the test, and of fresh and expired media at the 48-h renewal, and analysed for gibberellic acid by HPLC/UV. The limit of quantitation of the analytical method was 0.0360 mg a.s./L.
Observations:	Daily checks for mortality, sub-lethal effects.
Statistical analysis:	Not required.

II RESULTS AND DISCUSSION

A FINDINGS

Measured gibberellin concentrations in all samples taken from the test substance treatment ranged from 90% to 100% of nominal. The biological endpoints were expressed in relation to the mean measured concentrations of GA4/GA7.

There were no adverse effects among fish in the control group or any of the gibberellin treatment groups throughout the test.

B VALIDITY

The test was considered valid.

III CONCLUSIONS

The acute toxicity of gibberellins (GA4/GA7) to rainbow trout (*Oncorhynchus mykiss*) was determined in a 96-hour test under semi-static conditions. No treatment-related mortalities occurred. The 96-hour LC_{50} is > 96 mg a.s./L (mean measured), the highest concentration tested. In the absence of treatment-related mortality or behavioural abnormalities, the NOEC is considered to be 96 mg a.s./L.

RMS comments and conclusion:

The OECD 203 Fish acute toxicity test aims to determine toxicity of test substance to fish by determining LC_{50} at 24h, 48h, 72h and 96h.

Validity: For the test to be valid, the following performance criteria should be met:

- The mortality of the control should not exceed 10% (or one fish if less than 10 are used) at the end of

test **(in test: control mortality = 0%, condition fulfilled)**

- Constant conditions should be maintained as far as possible throughout the test and, if necessary, semi-static or flow-through procedures should be used **(in test: temperature from 13 to 16°C, dissolved oxygen from 6.2 to 9.7 mg/L, pH from 5.9 to 7.0, condition fulfilled)**

- The dissolved oxygen must have been at least 60% of the air saturation value throughout the test **(dissolved oxygen concentration of 6.2 mg/l represents 60% saturation at 14ºC in freshwater, in test: 6.2 to 9.7 mg/L, condition fulfilled)**

- There must be evidence that the concentration of the substance being tested has been satisfactory maintained and preferably it should be at least 80% of the nominal concentration throughout the test **(in test: from 90% to 100% of nominal value, condition fulfilled)**

The nominal GA4/GA7 concentrations were 6.3, 13, 25, 50 and 100 mg a.i./L. The mean measured concentrations were 5.7, 12, 25, 47 and 96 mg/L.

The endpoints should be expressed as nominal concentrations.

Acceptability of the analytical methods used in the test: /

Endpoints: The 96-hour LC_{50} is > 100 mg a.s./L (nominal), the highest concentration tested, the NOEC is 100 mg a.s./L.

Conclusion of the RMS: The endpoints were expressed as nominal concentration of the test substance. The test is considered valid.

b) Evaluation of additional data for the purpose of renewal of Annex I inclusion

No additional data was submitted or required for the purpose of renewal of approval of gibberellins GA4/GA7.

B.9.2.2. Long-term and chronic toxicity to fish

a) Previous evaluation (2005-2011)

Fish early life stage toxicity study was not submitted for the first inclusion of gibberellins GA4/GA7. A data gap was identified in EFSA conclusions (EFSA Journal 2012; 10(1):2502).

b) Evaluation of additional data for the purpose of renewal of Annex I inclusion

No data on the long-term and chronic toxicity to fish were submitted during the Annex I inclusion of gibberellins GA4/GA7. A new fish early life stage (ELS) toxicity test with GA3 is available, which is considered appropriate for the risk assessment of GA4/GA7 based on the similarities between GA3 and GA4/GA7, as well as the high margin of safety obtained in the risk assessment (see gibberellic acid DRAR Vol.3 CP Novagib B.9.4). This study with GA3 is therefore submitted for the purposes of renewal to avoid further unnecessary vertebrate testing, and is summarised below (CA 8.2.2.1/01) and referenced in DRAR Vol. 3 CA B.9.11.

Data point addressed:	CA 8.2.2.1/01
Author(s) (year):	[redacted] (2016)
Title:	Gibberellic acid (GA3): An early life-stage toxicity test with the Fathead Minnow (*Pimephales promelas*)
Laboratory report / project number:	[redacted]
Testing facility:	[redacted]
Published:	No
Test guideline used:	OECD 210; US EPA OPPTS 850.1400
Deviations:	Brief malfunctions of the air handler in the study room on Days 1, 2 and 21 of the test resulted in the temperature exceeding the target range of 25 ± 1 °C for short periods of time (approximately 1 to 1.5 hours) on these days. The temperature measurements made continuously in the negative control replicate A ranged from 23.19 to 27.23 °C. Since the deviations from the target temperature range were brief and had no noticeable impact on the health of the embryos or the larvae, this deviation from the protocol had no adverse impact on the results or the interpretation of the results of the study.
GLP:	Yes
Endpoint:	33 d NOEC (flow-through) = 11 mg a.s./L (mean measured)

Executive summary

Fathead minnows (*Pimephales promelas*) were exposed to gibberellic acid (GA3) at nominal test concentrations of 0.63 to 10 mg a.s./L (mean measured concentrations of 0.64 to 11 mg a.s./L) under flow-through conditions for 33 days (a 5-day hatching period plus a 28-day post-hatch growth period). There were no statistically significant treatment-related effects on hatching success or survival at concentrations ≤11 mg a.s./L. There were no biologically meaningful reductions in total length, wet weight or dry weight at concentrations ≤11 mg a.s./L. Consequently, the NOEC was determined to be 11 mg a.s./L and the LOEC to be >11 mg a.s./L.

I MATERIALS AND METHODS

A MATERIALS

Test item:	Gibberellic Acid (GA3) Technical powder
Lot No.:	237-979-S4
CAS No.:	77-06-5
Purity:	91.8 % a.i.
Expiry date:	January 2017
Description:	Solid
Stability of test compound:	Adequately stable under the conditions of the study. Samples of test media collected during the test performed under flow-through conditions had measured concentrations that ranged from 92.9% to 113% of nominal concentrations.

Test organism:	Fathead minnow (*Pimephales promelas*)
Source:	Embryos supplied by [redacted] received on spawning substrates
Age at test initiation:	<24 hours old

Temperature:	25 ± 1 °C*
Light intensity:	855 lux at water surface
Photoperiod:	16 h light; 8 h darkness. A 30-minute transition period of low light intensity was provided when lights went on and off to avoid sudden changes in lighting.
Dissolved oxygen:	≥ 88 % of saturation (7.2 mg/L)
pH:	8.1 - 8.3
Water hardness:	132 – 144 mg/L as $CaCO_3$

* The temperature measurements made continuously in the negative control replicate A ranged from 23.19 to 27.23°C. The measurements were slightly out of range for a short period of time on Days 1, 2 and 21 due to the failure of the air handler of the life cycle room where the study resided. Since the deviations from the target temperature range were brief and had no noticeable impact on the health of the embryos or the larvae, this deviation from the protocol had no adverse impact on the results or the interpretation of the results of the study.

B STUDY DESIGN

The study was conducted based on procedures outlined in OECD Guideline 210: *Fish, Early-life Stage Toxicity Test* (1); U.S. Environmental Protection Agency Series 850 – Ecological Effects Test Guidelines, OPPTS Number 850.1400: *Fish Early-Life Stage Toxicity Test* (2); and ASTM Standard E 1241-05: *Standard Guide for Conducting Early Life-Stage Toxicity Tests with Fishes* (3).

Fathead minnow embryos were exposed to a geometric series of five test concentrations, a negative (dilution water) control and a solvent control (0.1 mL/L HPLC-grade dimethylformamide) under flow-through conditions. The exposure period included a 5-day embryo hatching period, and a 28-day post-hatch juvenile growth period. Nominal test concentrations were 0.63, 1.3, 2.5, 5.0 and 10 mg a.s./L. Mean measured test concentrations were determined from samples of test water collected from each treatment and control group at the beginning of the test, at weekly intervals during the test and at test termination.

Delivery of the test solutions to the test chambers was initiated six days prior to test initiation in order to achieve equilibrium of the test substance. Four replicate test chambers were maintained in each treatment and control group, with one incubation cup in each test chamber. Each incubation cup contained 20 embryos, resulting in a total of 80 embryos per treatment. At test initiation, embryos <24 hours old were impartially distributed to incubation cups and exposed to test solution in the test chambers. After a 5-day embryo hatching period, the larvae were released into the test chambers, where exposure continued during a 28-day post-hatch juvenile growth period. Observations of the effects of gibberellic acid (GA3) on time to hatch, hatching success, growth, and survival were used to calculate the no-observed-effect-concentration (NOEC) and the lowest-observed-effect-concentration (LOEC). The effective concentrations for an x percent effect (ECx, typically EC10 and EC20) values with the 95% confidence intervals for hatching success, survival at the end of the test and growth (total length, wet weight and dry weight) endpoints and mean measured concentrations could not be estimated reliably because they exceeded the tested concentration range; therefore, the NOEC approach was used.

Water samples were collected from one test chamber of each treatment and control group two days prior to test initiation to confirm the operation of the diluter. Water samples were collected from alternating replicate test chambers of each treatment and control group on Days 0, 7, 14, 21, 28 and 33 (test termination) to determine concentrations of the test substance in the test chambers. On Days 0 and 7 of the test, all stock solutions were collected and analysed to confirm the test stock solutions. On Days 1 and 8 of the test stock solutions from the 50 and 13 mg a.s./mL secondary stocks, respectively, were collected and analysed to confirm the concentrations of these test stock solutions. All samples were collected at mid-depth, placed in glass vials containing an equal volume of 0.2% acidified (formic acid) methanol and processed immediately for analysis. Concentrations of gibberellic acid (GA3) in the samples were determined using an Applied Biosystems/MDS Sciex API 3000 LC/MS/MS coupled with an Agilent 1200 Series HPLC system. Chromatographic separations were achieved using a Thermo Betasil C-18 analytical column (50 mm x 2.1 mm, 5-µm particle size) and a Thermo Betasil C-18 guard column (10 mm x 2.1 mm).

II RESULTS AND DISCUSSION

A FINDINGS

Samples of the stock solutions being delivered to the diluter system had measured concentrations that ranged from 97.4 to 111% of nominal concentrations. The measured concentrations of samples collected to verify the diluter system prior to the test ranged from 101 to 115% of nominal concentrations. Samples of the test solutions collected during the test had measured concentrations that ranged from 92.9 to 113% of nominal concentrations. When the measured concentrations of test solution samples collected on Days 0, 7, 14, 21, 28 and 33 of the test were averaged for each treatment group, the mean measured test concentrations were 0.64, 1.3, 2.7, 5.2 and 11 mg a.s./L, which represented 102, 100, 108, 104 and 110% of nominal concentrations, respectively. The results of the study were based on the mean measured concentrations.

Daily observations of the embryos indicated that there were no apparent differences in time to hatch between the control groups and any of the gibberellic acid (GA3) treatment groups. The majority of fathead minnow embryos in the control and treatment replicates started to hatch on Days 4 and 5 of the test. Therefore, no statistical analysis was performed on time to hatch.

There were no statistically significant differences in hatching success between the negative and solvent control groups. Therefore, the control data were pooled for comparisons with the treatment groups. No statistically significant differences in hatching success were found in any of the treatment groups when compared to the pooled controls (Table 9.2.2/01- 1). Consequently, the NOEC for hatching success was 11 mg a.s./L and the LOEC was >11 mg a.s./L.

There were no statistically significant differences in larval survival between the negative and solvent control groups. Therefore, the control data were pooled for comparisons with the treatment groups. There were no statistically significant decreases in survival in any of the gibberellic acid (GA3) treatment groups in comparison to the pooled controls (Table 9.2.2/01- 1). Consequently, the NOEC for larval survival was 11 mg a.s./L and the LOEC was >11 mg a.s./L.

In general, the majority of the fish in the control and treatment groups appeared normal throughout the test. There were some organisms in the treatment groups that appeared lethargic, small, weak, discoloured (pale), having a loss of equilibrium, curled, lying on the bottom of the test chamber or noted with morphological deformity (i.e. crooked spine). However, these observations were infrequent and there were comparable observations in the controls.

There were no statistically significant differences in any of the growth parameters between the negative and solvent control groups. Therefore, the control data were pooled for comparisons with the treatment groups. Dunnett's one-tailed test indicated there was a statistically significant reduction in total length and dry weight among fish in the 1.3 and 11 mg a.s./L treatment groups and in wet weight among fish in the 1.3 mg a.s./L treatment group in comparison to the pooled controls (Table 9.2.2/01- 1). However, the reductions in total length, wet weight and dry weight in the 1.3 mg a.s./L treatment group did not follow a dose-response pattern, and were not considered to be biologically meaningful. Since the mean total length and dry weight of fish in the 11 mg a.s./L treatment group were comparable to those in the 1.3 mg a.s./L treatment group, the statistically significant reductions in total length and dry weight in the 11 mg a.s./L treatment group were also not considered to be biologically meaningful. Consequently, the NOEC for growth was 11 mg a.s./L and the LOEC was >11 mg a.s./L.

Table 9.2.2/01- 1 : Summary of hatching success, larval survival and growth of fathead minnows exposed to Gibberellic Acid (GA3)

Mean measured concentration (mg a.s./L)	% Hatching success[1,2]	% Survival to Day 28 post-hatch[1,2]	Growth parameters at Day 28 post-hatch[2]		
			Mean total length ± SD (mm)	Mean wet weight ± SD (mg)	Mean dry weight ± SD (mg)
Negative Control	95	80	25.4 ± 0.44	131 ± 5.99	25.6 ± 1.25
Solvent Control	91	88	25.1 ± 0.43	128 ± 4.63	24.0 ± 1.07
Pooled Control	93	84	25.2 ± 0.44	129 ± 5.20	24.8 ± 1.38
0.64	94	100	25.3 ± 0.068	128 ± 0.420	23.9 ± 0.446
1.3	98	85	24.7 ± 0.24[a,b]	123 ± 3.54[a,b]	22.1 ± 1.06[a,b]
2.7	98	99	25.2 ± 0.22	130 ± 4.78	23.7 ± 0.928
5.2	98	90	25.1 ± 0.22	132 ± 3.76	23.7 ± 0.797
11	98	91	24.7 ± 0.52 [a,c]	129 ± 4.95	23.0 ± 0.675[a,d]

[1] There were no significant reductions in hatching success or survival from the pooled control (Fisher's Exact test, $p>0.05$).

[2] The EC_x (i.e. EC_{10} and EC_{20}) and the corresponding 95% confidence interval for hatching success, survival and growth were not reported since the values were well above the concentrations tested.

[a] Indicates significant differences in growth from the pooled control (Dunnett's one-tailed test, $p\leq0.05$).

[b] Since the reductions in growth in the 1.3 mg a.s./L treatment group did not follow the dose-response pattern, the statistically significant reduction found was not a treatment related effect.

[c] Since the mean total length of the fish in the 11 mg a.s./L treatment group was the same as that of the 1.3 mg a.s./L treatment group, the reduction in total length found in the 11 mg a.s./L treatment group also was not considered to be treatment related.

[d] Since the mean dry weight of fish in the 11 mg a.s./L treatment group was the larger than that of the 1.3 mg a.s./L treatment group, the reduction in dry weight found in the 11 mg a.s./L treatment group also was not considered to be treatment related.

B VALIDITY

The test was considered valid as the following validity criteria were met:

- The dissolved oxygen concentration was >60% of the air saturation value throughout the test.
- The manual water temperature measurements made weekly during the test did not differ by more than ±1.5ºC between test chambers or between successive days at any time during the test, and were within the 25 ± 1°C range specified for the test species. However, brief malfunctions of the air handler in the study room on Days 1, 2 and 21 of the test, resulted in the temperature exceeding the target range for short periods of time (approximately 1 to 1.5 hours) on these days. The temperature measurements made continuously in the negative control replicate A ranged from 23.19 to 27.23°C. Since the deviations from the target temperature range were brief and had no noticeable impact on the health of the embryos or the larvae, this deviation from the protocol had no adverse impact on the results or the interpretation of the results of the study.
- The concentrations of the test substance in the solution were satisfactorily maintained within ±20% of the nominal concentration.
- The percent hatching success of fertilised eggs in the negative control group and in the solvent control group were > 70%, and the minimum percent post-hatch survival exceeded 75%.
- No significant effects on hatching success, survival or growth were observed in the solvent control.

III CONCLUSIONS

Fathead minnows (*Pimephales promelas*) were exposed to gibberellic acid (GA3) at mean measured concentrations of 0.64 to 11 mg a.s./L under flow-through conditions for 33 days (a 5-day hatching period plus a 28-day post-hatch growth period). There were no statistically significant treatment-related effects on hatching success or survival at concentrations ≤11 mg a.s./L. There were no biologically meaningful reductions in total

length, wet weight or dry weight at concentrations ≤11 mg a.s./L. Consequently, the NOEC was determined to be 11 mg a.s./L and the LOEC to be >11 mg a.s./L.

RMS comments and conclusion:

Tests with the early-life stages of fish are intended to define the lethal and sub-lethal effects of chemicals on the stages and species tested. They yield information of value for the estimation of the chronic lethal and sub-lethal effects of the chemical on other fish species.

Validity: According to the OECD 210 test guideline the early-life stage toxicity test is considered acceptable if the following validity criteria are met:

- the dissolved oxygen concentration should be >60% of the air saturation value throughout the test **(A dissolved oxygen concentration of 4.9 mg/L represents 60% saturation at 25ºC in freshwater, in test: between 7.9 ± 0.43 and 8.0 ± 0.0, condition fulfilled)**

- the water temperature should not differ by more than + 1.5°C between test chambers or between successive days at any time during the test, and should be within the temperature ranges specified for the test species (**for *Pimephales promelas* 25°C ± 1.5°C, in test: 23.19°C – 27.23°C, condition NOT fulfilled)**

- the analytical measure of the test concentrations is compulsory **(measured concentrations ranged from 92.9 to 113% of nominal concentrations, condition fulfilled)**

- overall survival of fertilised eggs and post-hatch success in the controls and, where relevant, in the solvent controls should be greater than or equal to the limits defined in Annex 2 **(for *Pimephales promelas*: hatching success > 70%, post-hatch success > 75%, in test: hatching success 91 – 98%, post-hatch success 80 – 100%, condition fulfilled)**

The temperature throughout the experiment was out of bounds. Absolute measured values ranged from 23.19°C to 27.23°C, with mean ± SD values ranged from 25.1 ± 0.70 °C to 25.2 ± 0.73°C. The RMS considers this to be a minor deviation that will have a major effect on validity of the study results.

Acceptability of the analytical methods used in the test: The analytical method for the determination of GA3 in freshwater is considered valid and acceptable according to SANCO/3029/99 rev.4. No further data required. The assessment is provided in Section B.5.1.2.6.

Endpoints: There were no biologically meaningful reductions in total length, wet weight or dry weight at concentrations ≤11 mg a.s./L (mean measured). Values EC_{10} and EC_{20} could not be determined. Consequently, the NOEC was determined to be 11 mg a.s./L and the LOEC to be >11 mg a.s./L.

Conclusion of the RMS: The fish early-life stage toxicity test is considered valid.

B.9.2.3. Bioconcentration in fish

The log Pow values of gibberellins GA4 and GA7 are 2.34 and 2.25, respectively, as agreed by EFSA (EFSA Journal 2012;10(1):2502). Given that the log Pow is lower than the trigger of 3 it is considered that there is no potential for bioaccumulation of GA4/GA7 in fatty tissues and therefore there is no potential for secondary

poisoning. Furthermore, GA4, GA7 and other gibberellins are naturally occurring (see CA 8.9/01, and the review of public domain literature on the presence of endogenous gibberellins in higher plants, algae, mosses and lichens as well as the biosynthesis of gibberellins by soil bacteria and fungi included at DRAR Vol. CA B.7). There is no evidence of bioconcentration in prey of birds and mammals from natural sources of GAs.

B.9.2.4. Potential for endocrine disruption

The EFSA/ECHA guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009 (EFSA Journal 2018;16(6):5311) foresees a gradual approach to identification of endocrine disrupting properties. It is recommended to strive for a conclusion on the ED properties with regard to humans and in parallel, using the same database, to strive for a conclusion on mammals as non-target organisms. Only where, based on this assessment, the criteria are not met for mammals as non-target organisms, would the assessment need to proceed to the other taxonomic groups. In order to conclude on the ED properties of gibberellins GA4/GA7 with regard to humans new data need to be generated. Until a conclusion is made regarding humans and other non-target mammals, it is unclear if assessment of ED properties of gibberellins GA4/GA7 needs to proceed to the other taxonomic groups. However, as some data regarding fish are available, the RMS attempted to assess the ED properties of gibberellins GA4/GA7 regarding fish. The assessment was performed in line with general assessment strategy described in ED GD (EFSA Journal 2018;16(6):5311) and summarised in scheme on page 13 of the ED GD (EFSA Journal 2018;16(6):5311). The assessment was done separately for EAS and T modalities.

1. *Gathering information*

Utilising the available ecotoxicological data for GA4/GA7, the applicant – European Gibberellin Task Force (EGTF, otherwise referred to as GA4/7 Task Force (GA4/7 TF)) has performed an evaluation of the potential for GA4/7 to be an endocrine disruptor according to the "Guidance for the Identification of Endocrine Disruptors in the Context of Regulations (EU) No. 528/2012 and (EC) No. 1107/2009" (EFSA Journal 2018; 16(6): 5311).

This evaluation comprises an assessment of the available literature data and an assessment of the ecotoxicological studies according to the abovementioned guidance utilising Appendix E1 and the corresponding sub-guidance document for completion of the Excel spreadsheet.

The applicant submitted results of a new literature search focused on endocrine disruption. This literature search has been performed additionally to the general literature search already performed. The general literature search included search term "endocrine disruptor", however, this is considered not sufficient. The additional literature search was done simultaneously for gibberellic acid (GA3) and gibberellins GA4/GA7. After a review of scientific peer-reviewed open literature no papers were identified that were relevant with respect to gibberellins GA4/GA7 and endocrine disruption. In the search 3 papers were found that were considered to be relevant for identification of endocrine disruptor properties of GA3, but only 1 was considered reliable. The applicant considers that based on structural similarity read-across of GA3 data to GA4/GA7 is acceptable. For literature search a single concept search strategy was utilized. The RMS found several shortcomings in the summary of the literature search. It is stated in the summary that literature search was performed covering the period from 1 April 2016 to 31 October 2018. In Table 2, where the search process is reported, a period from 1 April 2016 to 31 November 2017 is reported. Irrespective of which of the two periods was searched, both search periods are insufficient. The general literature search was performed for the period of the last 10 years and included the term "endocrine disruptor". This is insufficient. The additional literature search focused on endocrine disruption should also have been conducted for the past 10 years as instructed in the guidance document for identification of endocrine disruptors (EFSA Journal 2018; 16(6): 5311). In the literature search studies providing information that supports the existing regulatory data package were considered as non-relevant. The RMS is of the opinion

that this is not appropriate. The guidance document for identification of endocrine disruptors (EFSA Journal 2018; 16(6): 5311) states that all available evidence needs to be taken into consideration. This is especially important in cases where weight of evidence approach is used to reach a decision on whether or not a substance is an endocrine disruptor. One of the search terms reported in Table 2 is benalaxyl. As far as is known to the RMS, benalaxyl is a fungicide and has no connection to gibberellic acid or gibberellins. The following search terms were not used, but could have been: fecundity, fertility, sex ratio, egg, spiggin, vitellogenin, testis-ova, secondary sex characteristics, behaviour, aromatase, cyp19a1b, hatching. The RMS concludes that the literature search for endocrine disrupting properties of gibberellins GA4/GA7 in non-target organisms is inadequate.

The following amphibian study was identified in literature in the public domain. The study was conducted with gibberellic acid (GA3), not with gibberellins GA4/GA7. Based on structural similarity between GA3 and GA4/GA7 read-across of GA3 data to GA4/GA7 is acceptable. In view of its relevance to endocrine disruption the applicant included the study as supplementary information, however the study reported is not considered to be reliable for the reasons discussed below.

PREVIOUS EVALUATION	Not previously evaluated
Author(s) (year):	Sakr, S.A. and Shalaby, Y (2012)
Title:	Effect of gibberellin-A on metamorphosis in the Egyptian toad *Bufo regularis*
Citation:	The Journal of Basic and Applied Zoology **65**:153-156
Testing facility:	Department of Zoology, Faculty of Science, Menoufia University, Egypt
Published:	Yes
Test guideline used:	None
Deviations:	Not applicable; supplementary information from a non-standard study
GLP:	No

Summary

Two batches of 200 fertilised eggs were collected from wild-caught *B. regularis* and maintained in tap water under laboratory conditions. One group was treated with gibberellic acid, with the treatment maintained during the period of metamorphosis, the other served as the untreated control. Hatching success of eggs was recorded and external measurements were made of total body length and the length of the hind limbs and tails of tadpoles. Histological examinations were made of stained thyroid gland sections and measurements made of the diameter of follicles and follicular cell length. Lysed testicular tissue extracts were used to determine the presence of DNA laddering following agarose gel electrophoresis and gauge levels of apoptotic DNA fragmentation.

Egg hatch was 47.5% and 76.5% in the control and GA3-treated groups respectively. During the exposure phase the mean duration of metamorphosis (stages undefined) in tadpoles was 60 days in the control and shorter (52 days) in the GA3 treatment. Growth and development of tadpoles appeared slightly advanced in the GA3 animals, compared to the untreated controls, evidenced by differences of up to 2.3 mm in synchronous measurements of mean hind limb length and of up to 5 mm in mean total body length. Tails were absent in the GA3-exposed group after 52 days of treatment, but were still present in the control tadpoles up to 60 days. A higher degree of apoptotic DNA fragmentation was observed in the treated group compared to the control. Thyroid follicles were slightly (8%) larger in diameter and their cells longer (21%) in the GA3 treatment group compared to the control; only the difference in follicular cell length was statistically significant at $p<0.05$ and based on 15 determinations. The authors speculate that gibberellin-A3 acts as a thyroid hormone, resulting in the modified metamorphosis and apoptosis observed in this study.

Discussion

The closest analogous study type to that reported here is the AMA, OECD Test Guideline 231 adopted in 2009. Although OECD TG 231 is validated for a different anuran amphibian species (African clawed frog; *Xenopus laevis*), it does emphasise some key aspects of test design and procedure that require close attention to avoid artificially inducing effects on thyroid activity and metamorphosis. These factors are presumed to be broadly applicable to other frog and toad species, and therefore to be relevant to gauging the validity and reliability of the present study performed with *B. regularis*.

Whereas OECD TG 231 (§8) stresses a need for 'special attention' to ensure that the dilution water is free of substances associated with treated water supply infrastructure and water treatment processes, the Sakr & Shalaby (2012) study was conducted with a medium of 'tap water' apparently without prior conditioning. Substances specifically named by OECD TG 231 are copper, chloramine and chloramines which are toxic to anurans, and fluoride, perchlorate and chlorate ions that may interfere with the iodine transporter of the thyroid gland. No information is reported on these substances or water quality parameters, however only 47.5% egg hatch was recorded in the untreated control group (compared to 76.5% in the GA3 treatment), which points to a serious underlying deficiency in the test conditions and/or general health of the test animals. No evidence is provided to demonstrate that iodide availability matched the optimal range prescribed by OECD TG 231 (§9) to facilitate thyroid hormone (TH) synthesis by the thyroid gland, or that it was uniformly abundant in both treatments. A diet of cooked spinach[1] was provided once the hatched tadpoles began to feed. The authors provide no information on test design, replication and operating volume of test vessels per treatment or the biomass loading in terms of number of tadpoles per unit volume of test medium, while OECD TG 231 (§12) emphasises that high larval densities during the pre-exposure phase should be avoided to reduce the likelihood of impairing development during the testing phase. Gomez-Mestre *et al*. (2013)[2] provide evidence from studies with the western spadefoot toad (*Pelobates cultripes*) that the tadpole metamorphosis of some toads is accelerated during conditions (low water volume, high stocking density) that simulate the drying of seasonally ephemeral pond habitats. The observed acceleration was a response to elevated endogenous concentrations of corticosterone and thyroid hormone, coinciding with increased expression of the thyroid hormone receptor TRB. Similar findings of accelerated *Bufo melanostictus* tadpole metamorphosis in response to crowding are described by Saidapur and Girish (2001)[3], albeit without insight into the underlying endocrine mechanism.

The beginning of the exposure phase of the Sakr & Shalaby (2012) study is not defined in relation to the developmental stage of the test organisms. Events are variously reported in relation to 'age' in days (point of origin undefined) and days of treatment. Some measurements presented in tables and the corresponding text differ by three orders of magnitude. The test material is inadequately characterised. The exposure regime is not defined and the nominal 5 ppm GA3 treatment is ambiguously described as having been dosed for '*3 days/week for the period of metamorphosis*' and unsupported by confirmatory analysis. It is therefore unclear whether the test design was a semi-static regime in which medium renewals were performed on three occasions per week to maintain a constant nominal exposure, or whether the exposure concentration was ramped with a sequence of 5 ppm additions made 3×/week to a static system.

Test conditions were inadequate and the test design does not permit discrimination between effects ascribed to the test substance and artefacts potentially induced by any single one or combination of several conditions of the test. The low hatch rate in the control suggests that subsequent observed differences between the treated and untreated groups may at least partly result from a compromised control population. No indication is given as to

[1] Rationale not explained, but possibly intended as a dietary source of iodine. Moreover it should be noted that spinach represents a 'well known' source of phytoecdysteroids that may cause ED in insects and nematodes (Tarkowská, D. and Strnad, M. (2018). Isoprenoid-derived plant-signaling molecules: biosynthesis and biological importance. *Planta* **247**:1051-1066).

[2] Gomez-Mestre, I., Kulkarni, S. and Buchholz, D.R. (2013). Mechanisms and Consequences of Developmental Acceleration in Tadpoles Responding to Pond Drying. PLoS ONE 8(12): e84266. doi:10.1371/journal.pone.0084266.

[3] Saidapur, S.K. and Girish, S. (2001). Growth and Metamorphosis of Bufo melanostictus Tadpoles: Effects of Kinship and Density. *Journal of Herpectology*, **35**(2):249-254.

whether the tadpole density was thinned and equalised between treatments before the test animals reached key stages of development. The distinct possibility that the observed effects are an artefact of unequal population dynamics in the control and test treatments, and not related to exposure to GA3 at all, cannot be excluded. The authors' suggestion that the findings of this study indicate that GA3 acts as a thyroid hormone is not consistent with the weight of evidence provided by the mammalian toxicology dataset from regulatory standard, GLP-compliant studies performed with GA3, where an indication of endocrine activity via thyroid modality is overwhelmingly absent. In conclusion, although the study targeted parameters that are in principle relevant to the detection of thyroid-mediated developmental effects in amphibians, the study is technically deficient and unreliable.

RMS comments and conclusion:

This is a non GLP study. It was not performed according to any known OECD, US-EPA or ISO guidelines. The study is evaluated according to OECD TG 231 amphibian metamorphosis assay. The study was conducted with gibberellic acid (GA3), not with gibberellins GA4/GA7. Based on structural similarity between GA3 and GA4/GA7 read-across of GA3 data to GA4/GA7 is acceptable.

Validity: According to test guideline OECD 231 this test is considered valid if the following criteria are met:

1. Valid experiment in a test determined to be negative for thyroid activity:

- For any given treatment (including controls), mortality cannot exceed 10%. For any given replicate, mortality cannot exceed three tadpoles, otherwise the replicate is considered compromised
- At least two treatment levels, with all four uncompromised replicates, should be available for analysis
- At least two treatment levels without overt toxicity should be available for analysis

2. Valid experiment in a test determined to be positive for thyroid activity:

- Mortality of no more than two tadpoles/replicate in the control group can occur **(in test: not reported, condition not met)**

The study conclusion supported the hypothesis that gibberellic acid GA3 has an effect on thyroid activity, therefore the validity criteria under point 2. are applicable.

There are several deviations from OECD 231 test guideline:

- The study was performed with *Bufo regulari*, the test guideline supposes the use of *Xenopus laevis.*
- Only one test concentration of GA3 was used, this is less than 3 as required by OECD 231.
- The animals used for the experiment were collected in the wild. The test guideline requires larvae to be derived from in-house adults. It is impossible to predict what kind of environmental factors the animals were exposed to before being collected for the study. This makes them unsuitable for standardised testing.
- Only one replica was used for control and treatment. The test guideline requires 4 replicates.
- 200 eggs were used per treatment.
- It is unclear from the study report what kind of exposure system was used (flow-through, static renewal).
- Parameters of the exposure system are not reported (material used, volume, height of water, flow rate, temperature, pH, dissolved oxygen, hardness, alkalinity, conductivity).
- Analysis of water quality was not performed.
- Concentration of iodine in water is not reported.
- In the study eggs were exposed to treatment, the test guideline dictates that tadpoles should be exposed.
- Cooked spinach was fed to tadpoles. The OECD 231 requires Sera Micron®. The nutritional composition of cooked spinach is not reported.

- The study measured hatchability, total body length, hind limb length, tail length, DNA fragmentation, diameter of thyroid follicles and length of thyroid follicular cells. The OECD 231 requires measurement of mortality, hind limb length, snout to vent length (SVL), developmental stage, wet weight and thyroid histology.
- The hind limb length was not normalized to SVL, as required by OECD 231.
- MTC or HTC was not determined.
- The results were not discussed in relation to general toxicity.

Additionally, the results reported in Table 1 of the study report do not match the results described in the text. The table reports number of hatched embryos in control to be 95, the text reports 85, both numbers are said to represent 47.5%. The table reports number of hatched embryos in GA3 treated group to be 153, the text reports 14, both numbers are said to represent 76.5%. These inconsistencies reduce the reliability of this study.

Acceptability of the analytical methods used in the test: Not applicable.

Endpoints: Not determined.

Conclusion of the RMS: The study indicates possible effects of gibberellic acid (GA3) on amphibians. Based on structural similarity between GA3 and GA4/GA7 read-across of GA3 data to GA4/GA7 is acceptable. It is difficult to assess the relevance and reliability of the study because it was not conducted according to any known test guideline. The study greatly deviates from test guideline for amphibian metamorphosis OCED 231. The study has many deficiencies and could therefore be assessed as unreliable. However, being the only study on amphibians, the results of this study cannot be simply disregarded. This study alone is not sufficient to prove or disprove effects of GA3 (and by extension also effects of GA4/GA7) on amphibians.

The following terrestrial arthropod study was identified in literature in the public domain. The study was conducted with gibberellic acid (GA3), not with gibberellins GA4/GA7. Based on structural similarity between GA3 and GA4/GA7 read-across of GA3 data to GA4/GA7 is acceptable. The applicant included it as supplementary information; however the study reported is not considered to be reliable.

PREVIOUS EVALUATION	Not previously evaluated
Author(s) (year):	Neumann Visscher, S. (1980)
Title:	Regulation of grasshopper fecundity, longevity and egg viability by plant growth hormones
Citation:	*Experientia* **36**: 130-131
Testing facility:	Department of Biology, Montana State University, USA
Published:	Yes
Test guideline used:	None
Deviations:	Not applicable; supplementary information from a non-standard study
GLP:	No

Summary

Laboratory tests were performed on the premise that the reproductive physiology of univoltine phytophagous arthropods in temperate climates is correlated to the seasonal growth status and endogenous hormone levels of their host plants. The fecundity, egg viability and female longevity of the rangeland grasshopper *Aulocara eliotti* (Thomas) was known to be influenced by the growth status of its primary host plant, independently of the conditions under which the insect itself was reared. Since the seasonal growth status of the host plant was known to be influenced by the hormones GA3 (abundant in young plants) and abscisic acid (ABA) (abundant at senescence), experiments were performed to determine whether reproductive performance of the grasshopper may be influenced by the plant hormones present in its food.

Test design and findings

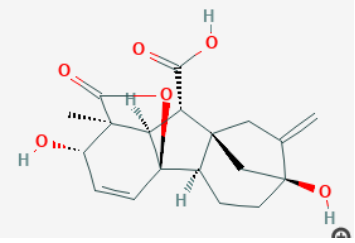
The test substance, GA3, sourced from Sigma Chemical Co, Grade III, G3250 was the technical active substance. GA3, dissolved initially in ethanol, then diluted with distilled water, at 60 and 600 mg/L was applied to the cut-grass diet of adult *A. elliotti* maintained in vials. The western wheatgrass (*Agropyron smithii*) that provided the diet as the primary host plant of the test organism was transplanted from a field site and maintained in a glasshouse under natural daylight and northern hemisphere summer (June – September) – *i.e*. pre-senescence - conditions. The test design included an untreated control with wheatgrass watered with distilled water, as well as additional treatments with the plant hormone abscisic acid (ABA), insect juvenile hormone JH III, and chloroform/methanol wheatgrass extracts that were thought likely to contain lipoid plant hormones, but without a corresponding solvent control. The additional treatments are not considered in detail here. Thirty pairs of test animals were initially assigned to each treatment, of these 13 to 25 pairs proved fertile.

Compared to the untreated control exposure to 60 and 600 mg GA3/L resulted in statistically significant reductions in fecundity, egg viability and rate of reproduction (numbers of eggs per adult per day) in *A. elliotti*, however full sets of results and indications of the degree of variability between replicates are not reported. In the GA3 treatments the reductions in the reproductive parameters relative to the water control were higher at the lower of the two dosing solution concentrations. The reduction in fecundity following treatment with 600 mg GA3/L was similar to that observed following exposure to 0.5 mg JH III/L.

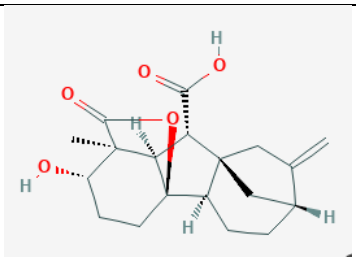
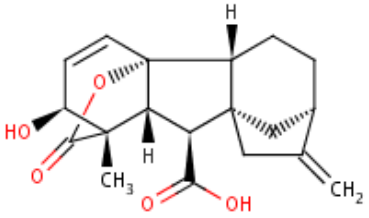
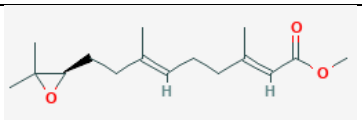
Discussion

The author speculates that reduced reproduction caused by the presence of the '*low*' concentrations of GA3 applied in this study may be a manifestation of impact on the test animals' metabolism via hormonal interference, claiming that GA3 and JH III are '*biochemically similar*' terpenoid compounds and are derived from a common precursor, mevalonate.

It is not possible to conclude that the effects observed in this study following exposure of *A. elliotti* to GA3 were caused by an endocrine mechanism. Moreover, according to more recent studies with the model plant *Arabidopsis*, the principal route of GA3 synthesis in plants occurs via the plastidial 2-C-methylerythritol-4-phospate (MEP) pathway (Kasahara *et al.*, (2002)[4]), so the premise of a common route of synthesis of GA3 and JH III via mevalonic acid (MVA) is incorrect. The structures of these substances (not reported) can be compared in the table below. The structures of GA3 and JH III have so few moieties and functional groupings in common (too few to support a credible QSAR read-across) that the suggested 'biochemical similarity' - and hence implied similarity of hormonal mode of action and effect between GA3 and JH III – is not convincing. The same applies for GA4/7 which are included in the table to facilitate comparison.

Substance	Structure
Gibberellic acid GA3	

[4] Kasahara, H., Hanada, A., Kuzuyama, T., Tagaki, M, Kamiya, Y. and Yamaguchi, S. (2002). Contribution of the mevalonate and methylerythritol phosphate pathways to the biosynthesis of gibberellins in Arabidopsis. *J. Biol. Chem.*, **277**:45188-45194. https://doi.org/10.1074/jbc.M208659200

Gibberellin GA4	
Gibberellin GA7	
Juvenile hormone JH III	

RMS comments and conclusion:

This is a non GLP study. It was not performed according to any known OECD, US-EPA or ISO guidelines. The study examined the effects of gibberellic acid GA3 on grasshopper (*Aulocara eliotti*). Based on structural similarity between GA3 and GA4/GA7 read-across of GA3 data to GA4/GA7 is acceptable.

Validity: Not applicable.

The results of the study indicate possible effects of gibberellic acid (GA3), abscisic acid (ABA) on fecundity and fertility of *Aulocara elliotti*. The authors hypothesised that plant hormones serve as biochemical signals to regulate insect reproduction and could be used as a safe control agent for phytophagous insects. The authors concluded that much more research is needed to confirm this hypothesis. While a recommendation for use of ABA as control agent was made, no conclusion was made regarding GA3.

The study has several deficiencies :

- Spray volume is not reported
- Mortality was not measured
- Ethanol was used for as solvent, but the content of ethanol in the final solutions is not reported
- No solvent control was used, only water control
- No dose response was observed in GA3 treatment
- Only 2 concentrations of GA3 treatment were used
- The animals used in experiment were collected in the wild
- Number of replicates is not reported
- No toxic reference standard was used
- Number of breeding pairs in different treatment groups ranged from 13 to 25
- Housing conditions are not reported
- Animals were fed cut grass treated with different treatments. Other conditions of exposure are not reported (number, interval, food consumption)

Acceptability of the analytical methods used in the test: Not applicable.

Endpoints: Not determined.

Conclusion of the RMS: The study is considered not reliable due to several deficiencies.

The following relevant terrestrial arthropod study was identified in literature in the public domain. The study was conducted with gibberellic acid (GA3), not with gibberellins GA4/GA7. Based on structural similarity between GA3 and GA4/GA7 read-across of GA3 data to GA4/GA7 is acceptable. The study is considered reliable and is included as supplementary information.

PREVIOUS EVALUATION	Not previously evaluated
Author(s) (year):	Abdellaoui, K. *et al*. (2013)
Title:	Biochemical and histological effects of gibberellic acid on *Locusta migratoria migratoria* fifth instar larvae
Citation:	Pesticide Biochemistry and Physiology **107**: 32-37
Testing facility:	Département des Sciences Biologiques et de la Protection des Végétaux, Institut Supérieur Agronomique de Chott-Mariem, Université de Sousse, Tunisia
Published:	Yes
Test guideline used:	None
Deviations:	Not applicable; supplementary information from a non-standard study
GLP:	No

Summary

Experiments were conducted to assess the effect of topical and oral administrations of gibberellic acid (GA3) on *Locusta migratoria migratoria* fifth instar larvae. Newly emerged larvae were exposed to various concentrations of GA3, administered by topical application or by oral intubation. Treated insects exhibited toxic symptoms with a dose-dependent mortality. GA3 toxicity was demonstrated by perturbation of the moult processes and an inability to complete ecdysis was observed to result in mortality of the 5th instar larvae. Histological examination of the proventriculus revealed alterations in the epithelial cells and the absence of apolysis. Exposure to GA3 also induced significant quantitative variation in haemolymph metabolites. These changes resulted in a significant decrease in the total concentration of proteins and carbohydrates and an increase in the total concentration of haemolymph lipids. The authors make reference to other literature in the public domain which postulates that adverse effects of GA3 treatment on several insect species may be linked to interference with endocrine processes because of a claimed structural similarity between GA3 and juvenile hormone (JH) in insects.

Test design and findings

The test substance, GA3, sourced from Sigma-Aldrich Chemie GmbH, batch and purity not stated, is presumed to have been the technical active substance. GA3 dissolved in acetone and distilled water was applied via both routes at concentrations of 0 (solvent control), 125, 625, 3125, 4125, 5125 and 6125 µg/mL (pH not reported), with 10 µL doses applied to the dorsal surface or administered by intubation directly into the proventriculus. Two experiments were performed with treatments applied either once only or repeatedly at 48 h intervals up to the final moult of the control insects. The total number of doses applied in the repeat-dose regime is not unambiguously reported. There were 30 newly emerged (0-1 day old) 5th instar larvae per treatment. Mortality was recorded and corrected for mortality in the corresponding control group. Haemolymph samples taken from the controls and the larvae of the repeatedly dosed 3125 and 6125 µg/mL groups of the contact and oral treatments were analysed for protein, carbohydrate and lipid content. Histological effects were examined proventriculus and fat body samples dissected from the orally-dosed insects after 8 days of treatment. Results were expressed as means ± standard deviations. Significant differences between treated groups and the relevant controls were identified by the Student-Newman-Keuls test at the 5% level and all data were analysed with SPSS (v 13.0) software.

Control group mortality rates are not reported. Corrected mortality in the groups treated once with GA3 was relatively low (<10% increasing to <20% in relation to dose), with the response to oral administration more sensitive than to the dermal contact exposure route. The repeated dose regime elicited higher dose-responsive corrected mortality, with the effect greater via oral than contact exposure; maximum corrected mortality at the 6125 µg/mL concentration was 65.3% (oral) and 48.1% (contact). Severe effects included incomplete emergence from the exoskeleton due to retention of the larval cuticle at the final moult ('causing mortality') and increased numbers of deformities among emerged adults. Histological examination revealed that the clear separation (exuvial space) between the old and new cellular intimae that is necessary to facilitate ecdysis and was evident in the control animals, had not properly developed in the groups exposed to GA3. Old cuticular intimae were contiguous with the intestinal epithelium and apolysis was absent in treated groups. Destruction of epithelial cells combined with disorganised cellular structures were features also noted in tissues from larvae exposed to GA3. Total protein content of the haemolymph was reduced in the GA3 treatments compared to the control group, with the highest effect observed at the highest application for both methods of exposure. Concentrations of total carbohydrate in the haemolymph were reduced in the GA3 treatments compared to the untreated control, with the effect more pronounced in the orally than the contact-dosed insects. Haemolymph lipid content increased in the GA3 treatments relative to the controls, corroborated by histological examinations of the dissected peripheral fat bodies. Whereas fat body cells in the control animals were rounded, with a nucleus and cytoplasm containing numerous colourless vacuoles confirmed by staining to be filled with lipid reserves, the fat cells in sections from treated locusts were smaller and more coloured and their lipidic droplets frequently appeared shrunken, presumably reflecting release of their content to haemolymph.

Discussion

The authors review the findings of this study in comparison with similar studies by the same and other workers in *L. migratoria* and other insect species exposed to GA3, noting a hypothesized association of observed effects with interference in endocrine metabolic processes involved with metamorphosis, based on a claimed similarity of '*chemical configuration*' (presumably structure) between the terpenoid GA3 and juvenile hormone (JH) and their common route of synthesis *via* the mevalonic acid pathway. However, this proposition appears simply to be a modification and an uncritical perpetuation of the one already cited by Neumann Visscher (1980), unsupported by any further confirmatory evidence or justification developed in the intervening >30 years. As already shown, a claim of similarity of chemical configuration between GA3 and JH cannot be substantiated. The exposure rates applied were high and whereas metamorphosis and ecdysis in arthropods is under hormonal control, it is not possible to conclude reliably that the effects observed in this study following exposure to GA3 were due primarily to endocrine disturbance rather than being secondary responses to systemic toxicity.

RMS comments and conclusion:

This is a non GLP study. It was not performed according to any known OECD, US-EPA or ISO guidelines. The study examined the effects of gibberellic acid GA3 on Migratory locust (*Locusta migratoria migratoria*). Based on structural similarity between GA3 and GA4/GA7 read-across of GA3 data to GA4/GA7 is acceptable.

Validity: Not applicable.

The following weaknesses were observed:

- Only solvent (ethanol) control was used, no negative (water) control.
- Animals were treated by forced ingestion via insulin syringe. The RMS considers this to be unusual and does not know how to evaluate this approach.
- Mortality of the control group was not reported. Only corrected mortality for treatment groups is reported.
- Number of replicates is not reported.
- No toxic reference standard was used.

Acceptability of the analytical methods used in the test: Not applicable.

Endpoints: Not determined.

Conclusion of the RMS: The study is acceptable as supporting information. The study indicates the potential effects of gibberellic acid GA3 on moulting and survival of Migratory locust. The moulting process is under hormonal regulation; therefore the effects of GA3 on moulting indicate possible protrusions of the hormonal signalling pathways in Migratory locust. Endocrine disrupting effects of chemicals on insects are not covered by the ED guidance document (EFSA Journal 2018; 16(6):5311), therefore the RMS concludes that this study can be used as supporting information.

The GA4/7 TF has conducted one *in vitro* study which included androgen and estrogen reporter gene assays. The assays are summarised below. No other ED specific studies were submitted.

(Anti)Estrogenic Evaluation

Study title	Reporter gene assays for gibberellic acid (GA4/A7) using human estrogen and androgen receptors.
Reference/Study number	Saito K (2008). Study number UKT-0038.
Testing facility	Environmental Health Science Laboratory, Sumitomo Chemical Co., Ltd, 1-98, 3-Chome, Kasugade-Naka, Konohana-Ku, Osaka, Japan.
Data point addressed	KCA 5.8.3
Test material	Gibberellic acid mixture (GA4/A7*) (60.4 %/30.2 % purity, respectively [indicating 90.6% purity of the mixture and a 2:1 ratio of GA4:GA7]). * GA4: "(1.alpha.,2.beta.,4a.alpha.,4b.beta.,1O.beta.) 1,4a-Jactone 2,4a-dihydroxy-1-methyl-8-methylene gbbane-1,10-dicarboxylic acid" [sic] GA7: (1.alpha.,2.beta.,4a.alpha.,4b.beta.,1O.beta.)-1,4a-lactone 2,4a-dihydroxy-1-methyl-8- methylene gibb-3-ene-1,10- dicarboxylic acid)
Test material source	Valent Biosciences Corporation, IL, USA.
Study type	Mammalian cell-based luciferase reporter gene assays.
Species	Human HeLa (cervical cancer) cell line with receptor expression vectors pRc/RSV-hERα and reporter plasmids [luciferase reporter vectors] pGL3-TATA-EREx5.
Route of exposure	In vitro.
Duration of exposure	40 hours.
Dose/concentrations tested	0 nM, 1 nM, 10 nM, 100 nM, 1 µM, 10 µM.
Test conditions	Modified human cells (HeLa cervical cancer cell line) expressing the human estrogen receptor α (hERα) were exposed to concentrations of 0 nM, 1 nM, 10 nM, 100 nM, 1 µM or 10 µM gibberellic acid (GA4/A7, ratio 2:1) in the presence or absence of estrogen receptor-active 17 β-estradiol (E2). Each concentration was tested in six independent experiments. Anti-estrogenic effects were determined in the presence of receptor competitor E2; estrogenic effects were determined in its absence. Differences were evaluated for statistical significance ($p<0.05$) using the t-test computed by Excel (Microsoft). Note: The androgenic aspects of this study are discussed in a separate summary.
Solvent	DMSO [dimethylsulfoxide].
Controls	Solvent and positive (estrodiol (E2) and 4-hydroxytamoxifen (HTM)) controls were employed for comparative purposes.
Parameters measured	Luciferase induction (as a proxy for activity at the receptor).
Test guideline	No guideline specified. The most relevant OECD Test Guideline would be OECD TG 455 – Performance-Based Test Guideline for Stably Transfected Transactivation In Vitro Assays to Detect Estrogen Receptor Agonists and Antagonists. This guideline was updated and adopted in 2016, whilst the study was performed in 2008.
Guideline deviations	Main deviations from OECD Guideline 455 (2016): Only one positive control substance (17 β-estradiol, E2) and one solvent control substance (DMSO)were employed for the agonist assay; whilst for the antagonist assay, only one positive control substance was employed (4-hydroxytamoxifen). The guideline recommends verifying the responsiveness of the test system by employing, with acceptability criteria, E2 and 17α-methyltestosterone as positive control substances and corticosterone as a negative control substance for the agonist assay, and tamoxifen as a positive control substance and flutamide as a negative control substance for the antagonist assay.

	The maximum concentration tested was 10 µM. The guideline recommends testing a maximum concentration, in the absence of precipitation or cytotoxicity, of 1 mM. Test substance precipitation was not given in the study report and there was no evidence of cytotoxicity in the concentration range tested. The report provides no information regarding testing the modified cell line for stability. The guideline recommends reference standard substances for monitoring cell line stability for both the antagonism and agonism assays. The cells were maintained in phenol red-free Dulbecco's Modified Eagle Medium (DMEM) containing 10 % charcoal-treated foetal calf serum. The guideline recommends phenol red free Eagle's Minimum Essential Medium (EMEM) supplemented with 10 % dextran-coated charcoal-treated foetal bovine serum and kanamycine (60 mg/L). Incubation conditions differed from those outlined in the guideline. Although there are a number of methodological deviations from the current OECD Guideline, this study was performed 8 years prior to its publication. It is considered that the data within the study and the conclusions drawn however, are scientifically credible and suitably robust for an assessment of GA4/A7's potential to be (anti)estrogenic.
GLP compliance	Not reported.
Acceptability of results	Klimisch score 2 (reliable with restrictions). There are deviations from OECD 455 (2016), but the results and conclusions are considered robust and reliable.
Conclusion	The investigators concluded that GA4/A7 shows no estrogenic or anti-estrogenic potential under the conditions of this study.

Executive Summary

In a mammalian cell-based luciferase reporter gene assay to evaluate the endocrine active potential of gibberellic acid (GA4/A7 mixture, ratio 2:1), human cells, (HeLa immortal cervical cancer cell line), modified to express the human estrogen receptor α were exposed to concentrations of up to 10 µM GA4/A7 in the presence and absence of an estrogen receptor competitor. A lack of test substance attributable effects in the assays led the investigators to conclude that GA4/A7 lacks estrogenic or anti-estrogenic potential under the conditions of this study.

Results and Discussion

GA4/A7 produced no statistically significant or notable effects on luciferase induction (a proxy for receptor activation or antagonism) at any of the concentrations tested.

It was not active at the human estrogen receptor α (hERα), as shown by a lack of ability to induce luciferase.

In the competitive binding assays, it did not reduce the induction of luciferase by the relevant endocrine active chemical (estradiol, E2), indicating a lack of antagonistic activity and, by extension, a lack of anti-estrogenic potential.

GA4/A7 did not show cytotoxicity or trans-activational activity in a receptor-independent manner in either of the assays.

RMS comments and conclusion:

The study was not performed according to any known guideline. The study is similar to test guideline OECD 455 Performance-based test guideline for stably transfected transactivation in vitro assays to detect estrogen receptor agonists and antagonists.
Deviations :

- Cell line HeLa9903 is recommended by the OECD 455. In this study Human HeLa (cervical cancer) cell line with receptor expression vectors pRc/RSV-hERα and reporter plasmids [luciferase reporter vectors] pGL3-TATA-EREx5 was used. This cell line is not validated for stability and integrity.
- It is not reported if cell line was tested for mycoplasma infection
- E2, 17α-estradiol, 17α-methyltestosterone and corticosterone should be used as the reference standards for agonist assay. Tamoxifen and flutamide should be used as reference standards for antagonist assay. In this study only E2 and 4-hydroxytamoxifen were used.
- The passage of cell line is not reported.
- Medium DMEM was used instead of EMEM.
- Cells were seeded with density $2x10^4$, instead of $1x10^4$.
- The sensitivity of the test system has not been tested beforehand.
- The vehicle used was DMSO. It was not demonstrated that DMSO does not interfere with assay performance.
- The highest tested concentration was 10µM, the test guideline requires 1mM.
- Potential solubility issues (cloudiness) are not reported.
- Cytotoxicity of the test chemical was not investigated. The test guideline states: Should the results of the cytotoxicity test show that the concentration of the test chemical has reduced the cell number by 20% or more, this concentration should be regarded as cytotoxic, and the concentrations at or above the cytotoxic concentration should be excluded from the evaluation.
- The edge effect was not excluded.
- Preparation of test chemicals is not described (dilution process, volume of the test chemical added to each well, final volume in well).
- The exposure to test chemicals was 40h; the test guideline dictates 20-24h exposure.

The study report states that n=6 for each treatment in this study. It is the understanding of the RMS that this usually means 6 measurements, in the case of this study 6 replicates is equivalent to 6 wells on 96 well plate. The applicant states in the study report that each concentration was tested in 6 independent experiments. Having 6 replicates is not the same as having 6 experiments.

Conclusion of the RMS: The study indicates that gibberellins GA4/GA7 do not bind to estrogen receptor; however it is difficult to assess the reliability of the study because it was not conducted according to any known test guideline. The study deviates from test guideline OECD 455. The RMS considers that this study alone is not sufficient to show that GA4/GA7 do not bind to estrogen receptor. The study is considered as supporting information.

(Anti)Androgenic Evaluation

Study title	Reporter gene assays for gibberellic acid (GA4/A7) using human estrogen and androgen receptors.
Reference/Study number	Saito K (2008). Study number UKT-0038.
Testing facility	Environmental Health Science Laboratory, Sumitomo Chemical Co., Ltd, 1-98, 3-

	Chome, Kasugade-Naka, Konohana-Ku, Osaka, Japan.
Data point addressed	KCA 5.8.3
Test material	Gibberellic acid mixture (GA4/A7*) (60.4%/30.2% purity, respectively [indicating 90.6% purity of the mixture and a 2:1 ratio of GA4:GA7]). * GA4: "(1.alpha.,2.beta.,4a.alpha.,4b.beta.,1O.beta.) 1,4a-Jactone 2,4a-dihydroxy-1-methyl-8-methylene gbbane-1,10-dicarboxylic acid" [sic] GA7: (1.alpha.,2.beta.,4a.alpha.,4b.beta.,1O.beta.)-1,4a-lactone 2,4a-dihydroxy-1-methyl-8- methylene gibb-3-ene-1,10- dicarboxylic acid)
Test material source	Valent Biosciences Corporation, IL, USA.
Study type	Mammalian cell-based luciferase reporter gene assays.
Species	Human HeLa (cervical cancer) cell line with receptor expression vectors RSV-hAR and reporter plasmids [luciferase reporter vectors] pGL3-ARE.
Route of exposure	In vitro.
Duration of exposure	40 hours.
Dose/concentrations tested	0 nM, 1 nM, 10 nM, 100 nM, 1 µM, 10 µM.
Test conditions	Modified human cells (HeLa cervical cancer cell line) expressing the human androgen receptor (hAR) were exposed to concentrations of 0 nM, 1 nM, 10 nM, 100 nM, 1 µM or 10 µM gibberellic acid (GA4/A7, ratio 2:1) in the presence or absence of androgen receptor active dihydrotestosterone (DHT). Each concentration was tested in six independent experiments. Anti-androgenic effects were determined in the presence of receptor competitor DHT, with androgenic effects determined in its absence. Differences were evaluated for statistical significance ($p<0.05$) using the t-test computed by Excel (Microsoft). Note: The estrogenic aspects of this study are discussed in a separate summary.
Solvent	DMSO [dimethylsulfoxide].
Control	Solvent and positive (dihydrotestosterone (DHT) and hydroxyflutamide (HFT)) controls were employed for comparative purposes.
Parameters measured	Luciferase induction (as a proxy for activity at the receptor).
Test guideline	No guideline specified. The most relevant OECD Test Guideline would be OECD TG 458 – Stably Transfected Human Androgen Receptor Transcriptional Activation Assay for Detection of Androgenic Agonist and Antagonist Activity of Chemicals. This guideline was adopted in 2016, whilst the study was performed in 2008.
Guideline deviations	Main deviations from OECD Guideline 458 (2016): Only one positive control substance (dihydrotestosterone, DHT) and one solvent control substance (DMSO) were employed for the agonist assay; whilst for the antagonist assay, only one positive control substance was employed (hydroxyflutamide, HFT). The guideline recommends verifying the responsiveness of the test system by employing, with acceptability criteria, DHT and mestanolone as positive control substances for the agonist assay, HF and Bisphenol A (BPA) as positive control substances for the antagonist assay and di(2-ethylhexyl)phthalate (DEHP) as a negative control substance for both assays. The cell line employed was of human origin. The guideline recommends the AR-EcoScreen cell line, which is derived from the Chinese hamster ovary cell line CHO-K1. The maximum concentration tested was 10 µM. The guideline recommends testing a "limit dose", in the absence of precipitation, of 1 mM. No information regarding test substance precipitation was given in the study report and there was no evidence of

	cytotoxicity in the concentration range tested. The report provides no information regarding testing the modified cell line for stability. The guideline recommends reference standard substances for monitoring cell line stability for the AR antagonism and agonism assays. The cells were maintained in phenol red-free Dulbecco's Modified Eagle Medium (DMEM) containing 10% charcoal-treated foetal calf serum. The guideline recommends phenol red-free D-MEM/F-12 (a medium containing Ham's F-12 nutrient mixture) supplemented with 5 % dextran-coated charcoal treated foetal bovine serum, penicillin (100 units/mL) and streptomycin (100 µg/mL). Incubation conditions differed from those outlined in the guideline. Although there are a number of methodological deviations from the current OECD Guideline, this study was performed 8 years prior to its publication. It is considered that the data within the study and the conclusions drawn however, are scientifically credible and suitably robust for an assessment of GA4/A7's potential to be (anti)androgenic.
GLP compliance	Not reported.
Acceptability of results	Klimisch score 2 (reliable with restrictions). There are deviations from OECD 458 (2016), but the results and conclusions are considered robust and reliable.
Conclusion	The investigators concluded that GA4/A7 shows no androgenic or anti-androgenic potential under the conditions of this study.

Executive Summary

In a mammalian cell-based luciferase reporter gene assays to evaluate the endocrine-active potential of gibberellic acid (GA4/A7 mixture, ratio 2:1), human cells (HeLa immortal cervical cancer cell line), modified to express the human androgen receptor, were exposed to concentrations of up to 10 µM GA4/A7 in the presence and absence of an androgen receptor competitor. A lack of test substance attributable effects in the assays led the investigators to conclude that GA4/A7 lacks androgenic or anti-androgenic potential under the conditions of this study.

Results and Discussion

GA4/A7 produced no statistically significant or notable effects on luciferase induction (a proxy for receptor activation or antagonism) at any of the concentrations tested.

It was not active at the human androgen receptor (hAR), as shown by a lack of ability to induce luciferase.

In the competitive binding assays it did not reduce the induction of luciferase by the relevant endocrine active chemical (dihydrotestosterone, DHT), indicating a lack of antagonistic activity and, by extension, a lack of anti-androgenic potential.

GA4/A7 did not show cytotoxicity or trans-activational activity in a receptor independent manner in either of the assays.

RMS comments and conclusion:

The study was not performed according to any known guideline. The study is similar to test guideline OECD 458 Stably Transfected Human Androgen Receptor Transcriptional Activation Assay for Detection of Androgenic Agonist and Antagonist Activity of Chemicals.
Deviations :

- Cell line AR-EcoScreen™ is recommended by the OECD 458. AR-EcoScreen™ is derived from Chinese hamster ovary cell line (CHO-K1). In this study human HeLa (cervical cancer) cell line with receptor expression vectors pRc/RSV-hAR and reporter plasmids [luciferase reporter vectors] pGL3-ARE was used. This cell line is not validated for stability and integrity.
- It is not reported if cell line was tested for mycoplasma infection
- Dihydrotestosterone (DHT), mestanolone and di(2-ethylhexyl)phthalate (DEHP) should be used as the reference standards for agonist assay. Hydroxyflutamide (HF), bisphenol A (BPA) and DEHP should be used as reference standards for antagonist assay. In this study only DHT and HF were used.
- The passage of cell line is not reported.
- Medium DMEM was used instead of DMEM/F2.
- Cells were seeded with density $2x10^4$, instead of $1x10^5$.
- The sensitivity of the test system has not been tested beforehand.
- The vehicle used was DMSO. It was not demonstrated that DMSO does not interfere with assay performance.
- The highest tested concentration was 10µM, the test guideline requires 1mM (if solubility allows).
- Potential solubility issues (cloudiness) are not reported.
- Cytotoxicity of the test chemical was not investigated. The test guideline states: Should the results of the cytotoxicity test show that the concentration of the test chemical has reduced the cell number by 20% or more, this concentration should be regarded as cytotoxic, and the concentrations at or above the cytotoxic concentration should be excluded from the evaluation.
- The edge effect was not excluded.
- Preparation of test chemicals is not described (dilution process, volume of the test chemical added to each well, final volume in well).
- The exposure to test chemicals was 40h; the test guideline dictates 20-24h exposure.

The study report states that n=6 for each treatment in this study. It is the understanding of the RMS that this usually means 6 measurements, in the case of this study 6 replicates is equivalent to 6 wells on 96 well plate. The applicant states in the study report that each concentration was tested in 6 independent experiments. Having 6 replicates is not the same as having 6 experiments.

Conclusion of the RMS: The study indicates that gibberellins GA4/GA7 do not bind to androgen receptor; however it is difficult to assess the reliability of the study because it was not conducted according to any known test guideline. The study deviates from test guideline OECD 458. The RMS considers that this study alone is not sufficient to show that GA4/GA7 do not bind to androgen receptor. The study is considered as supporting information.

For assessment of endocrine disrupting properties of gibberellins GA4/GA7 one *in vitro* study is available (see Table 9.2.4 - 1), which RMS considers to be unreliable and is suggested to be used as supporting information (UKT-0038). Additionally one guideline study with gibberellic acid (GA3) is available; Fish early life stage toxicity test (OECD 210). The study does not provide any EATS-mediated parameters, *in vivo* mechanistic parameters or *in vitro* mechanistic parameters. The study provides information on Sensitive to but not diagnostic of EATS parameters. Based on structural similarities between GA3 and GA4/GA7 read across approach is acceptable.

Table 9.2.4 - 1: Available information for assessment of ED properties of GA4/GA7 regarding fish

Species	Endpoint	Value [mg/L]	Study ID (Appendix E) / Year)	Laboratory report / project number
Pimephales promelas (ELS study) – study conducted with GA3	33 d NOEC	> 11 mg/L	6 / 2016	[redacted]
Stably Transfected Human AR and ERα Transactivation Assay	-	-	5 / 2008	UKT-0038

2. Assessment of the evidence

The applicant extracted and reported the information in the form of an Excel spreadsheet. The Excel spreadsheet is provided in Appendix 1 of this document.

a. EAS modality

Assembled lines of evidence are presented below in Table 9.2.4 - 2. Lines of evidence for general toxicity are from the study conducted with gibberellic acid (GA3). Based on structural similarities between GA3 and GA4/GA7 read across approach is acceptable.

Table 9.2.4 - 2: Lines of evidence for EAS modalities.

	Study ID Matrix	Effect classification	Effect target	Species	Duration of exposure	Duration unit	Route of administration	Lowest Effect dose	Dose unit	Effect direction	Observed effect (positive and negative)	Assessment of each line of evidence	Assessment on the integrated line of evidence	Modality
Integrated LoE for endocrine activirty	5	In vitro mechanistic	Androgen receptor	Human	40	Hours	Uptake from the medium	>10000	other	No effect	No effect	Supporting information. The study was assessed as not reliable.	Overall not sufficient to show absence of endocrine activity.	A
	5	In vitro mechanistic	Estrogen receptor	Human	40	Hours	Uptake from the medium	>10000	other	No effect	No effect	Supporting information. The study was assessed as not reliable.		E
LoE for general toxicity	6	Sensitive to, but not diagnostic of, EATS	Behaviour (fish)	Fathead minnow	33	days	Uptake from water	>11	mg/L water	No effect	No effect	Not sufficient. No effects observed, but only one study performed, only one species.	Overall indicates absence of general toxicity. Considered not sufficient to show absence of adversity.	N
	6	Sensitive to, but not diagnostic of, EATS	Body weight (fish)	Fathead minnow	33	days	Uptake from water	>11	mg/L water	No effect	No effect	Not sufficient. No effects observed, but only one study performed, only one species.		N
	6	Sensitive to, but not diagnostic of, EATS	Embryo time-to-hatch	Fathead minnow	33	days	Uptake from water	>11	mg/L water	No effect	No effect	Not sufficient. No effects observed, but only one study performed, only one species.		N
	6	Sensitive to, but not diagnostic of, EATS	Length (fish)	Fathead minnow	33	days	Uptake from water	>11	mg/L water	No effect	No effect	Not sufficient. No effects observed, but only one study performed, only one species.		N

	6	Sensitive to, but not diagnostic of, EATS	Survival of embryos	Fathead minnow	33	days	Uptake from water	>11	mg/L water	No effect	No effect	Not sufficient. No effects observed, but only one study performed, only one species.		N
	6	Systemic toxicity	Survival (fish)	Fathead minnow	33	days	Uptake from water	>11	mg/L water	No effect	No effect	Not sufficient. No effects observed, but only one study performed, only one species.		N

The available evidence is considered not sufficient to show absence of endocrine activity or adversity.

b. T modality

Assembled lines of evidence for T modality are presented below in Table 9.2.4 - 3. Lines of evidence for general toxicity are from the study with gibberellic acid (GA3). Based on structural similarities between GA3 and GA4/GA7 read across approach is acceptable.

Table 9.2.4 - 3 : Lines of evidence for T modality

	Study ID Matrix	**Effect classification**	**Effect target**	**Species**	**Duration of exposure**	**Duration unit**	**Route of administration**	**Lowest Effect dose**	**Dose unit**	**Effect direction**	**Observed effect (positive and negative)**	**Assessment of each line of evidence**	**Assessment on the integrated line of evidence**	**Modality**
LoE for general toxicity	6	Sensitive to, but not diagnostic of, EATS	Behaviour (fish)	Fathead minnow	33	days	Uptake from water	>11	mg/L water	No effect	No effect	Not sufficient. No effects observed, but only one study performed, only one species.	Overall indicates absence of general toxicity. Considered not sufficient to show absence of adversity.	N
	6	Sensitive to, but not diagnostic of, EATS	Body weight (fish)	Fathead minnow	33	days	Uptake from water	>11	mg/L water	No effect	No effect	Not sufficient. No effects observed, but only one study performed, only one species.		N

	6	Sensitive to, but not diagnostic of, EATS	Embryo time-to-hatch	Fathead minnow	33	days	Uptake from water	>11	mg/L water	No effect	No effect	Not sufficient. No effects observed, but only one study performed, only one species.		N
	6	Sensitive to, but not diagnostic of, EATS	Length (fish)	Fathead minnow	33	days	Uptake from water	>11	mg/L water	No effect	No effect	Not sufficient. No effects observed, but only one study performed, only one species.		N
	6	Sensitive to, but not diagnostic of, EATS	Survival of embryos	Fathead minnow	33	days	Uptake from water	>11	mg/L water	No effect	No effect	Not sufficient. No effects observed, but only one study performed, only one species.		N
	6	Systemic toxicity	Survival (fish)	Fathead minnow	33	days	Uptake from water	>11	mg/L water	No effect	No effect	Not sufficient. No effects observed, but only one study performed, only one species.		N

The available evidence is considered not sufficient to show absence of endocrine activity or adversity.

3. Initial analysis if evidence

The RMS attempted to perform the initial analysis of evidence for endocrine disruption properties of GA4/GA7 in accordance with the decision tree presented in the Figure 1 of the ED guidance document (EFSA Journal 2018; 16(6):5311). This step of the assessment includes a decision tree with different possible scenarios. Which scenario is relevant is decided based on whether either EATS-mediated adversity or EATS endocrine activity has been sufficiently investigated. For the GA4/GA7 data package the assessment is the following:

Table 9.2.4 - 4 : Question from decision tree

Question in decision tree	Answer	Explanation
Have EATS mediated parameters been sufficiently investigated?	No	For the EATS mediated parameters in non-target organisms to be considered sufficiently investigated the studies OECD TG 240 (or US EPA 850.1500) and OECD TG 231 (or OECD TG 241) should have been conducted.
Has EATS-mediated adversity been observed?	No	
Has endocrine activity been observed?	No	
Has endocrine activity been sufficiently investigated?	No	For the endocrine activity to be considered sufficiently investigated the studies OECD TG 229 (or OECD TG 230) and OECD TG 231 should have been conducted. The GA4/GA7 data package includes studies conducted with GA3. Based on structural similarity between GA3 and GA4/GA7 read-across of GA3 data to GA4/GA7 is acceptable. The data package provided by the applicant contained one *in vitro* study (UKT-0038) which investigated the effects of GA4/GA7 on estrogen and androgen receptor. The study was evaluated as supporting information. This means that endocrine activity was not sufficiently investigated for E and A modalities. The T (thyroid) and S (spermatogenesis) modalities were not investigated.

The decision tree in Figure 1 of the guidance document (EFSA Journal 2018; 16(6):5311) guides the assessment to Scenario 2a(iii). New data need to be generated to be able to assess the endocrine disrupting properties of GA4/GA7 for ecotoxicological assessment.

Table 9.2.4 - 5: Selection of relevant scenario

Adversity based on EAS-mediated parameters	**Positive mechanistic OECD CF level 2/3 Test**	**Scenario**	**Next step of the assessment**	**Scenario selected** *(indicate with an "x" the scenario selected based on the assessed lines of evidence)*
No (sufficiently investigated)	Yes/No	1a	Conclude: ED criteria not met because there is no "**EATS-mediated**" adversity	
Yes (sufficiently investigated)	Yes/No	1b	Perform MoA analysis	
No (not sufficiently investigated)	Yes	2a (i)	Perform MoA analysis (additional information may be needed for the analysis)	
No (not sufficiently investigated)	No (sufficiently investigated)	2a (ii)	Conclude: ED criteria not met because no **EATS-mediated endocrine activity** observed	
No (not sufficiently investigated)	No (not sufficiently investigated)	2a (iii)	Generate missing level 2 and 3 information. Alternatively, generate missing "**EATS-mediated**" parameters. Depending on the outcome move to corresponding scenario	**X**
Yes (not sufficiently investigated)	Yes/No	2b	Perform MoA analysis	

To investigate endocrine activity the following studies need to be performed:
Level 2:

- Performance-based test guideline for stably transfected transactivation in vitro assays to detect estrogen receptor agonists and antagonists (OECD 455) to investigate E modality
- Stably Transfected Human Androgen Receptor Transcriptional Activation Assay for Detection of Androgenic Agonist and Antagonist Activity of Chemicals (OECD 458) to investigate A modality
- Steroidogenesis *in vitro* (OECD TG 456) or Aromatase assay (US EPA TG OPPTS 890.1200) to investigate S modality

Level 3:

- Amphibian metamorphosis assay (OECD TG 231) to investigate T modality
- Fish short term reproduction assay (OECD TG 229) to investigate EAS modalities

To investigate adversity the following studies need to be performed:
Level 3:

- Amphibian metamorphosis assay (OECD TG 231) to investigate T modality (already mentioned above)

Level 5:

- Medaka extended one generation reproduction test (OECD TG 240) to investigate EAS modalities

4. Conclusion regarding ED properties in fish and amphibians

Based on the available evidence from standard studies for non-target organisms, the EATS-modalities are not considered sufficiently investigated. The dataset is not sufficient to assess the ED properties of gibberellins GA4/GA7. According to the assessment strategy of the guidance for the identification of endocrine disruptors (ECHA, EFSA 2018), a tiered assessment strategy should be followed. In the case of gibberellins GA4/GA7, level 2 and level 3 tests would be required to complete the current data package:

1. A study in line with the OECD 455 (estrogen receptor transactivation)
2. A study in line with the OECD 458 (androgen receptor transactivation)
3. A study in line with the OECD 456 (steroidogenesis)
4. A study in line with the OECD TG 231 (AMA)
5. A study in line with the OECD TG 229 (FSTRA)

These tests investigate potential EATS-mediated endocrine activity. If all tests are negative, this shows that gibberellins GA4/GA7 have no ED properties. However, if these tests show a positive result for at least one modality, additional testing might be needed in order to further investigate the adversity.

In order to be able to conclude whether the approval criteria on the endocrine disruption potential in line with Commission Regulation (EU) 2018/605[5] are met for gibberellins GA4/GA7, the applicant should complete the data package within a period not exceeding 30 months. However, the decision whether or not to request the listed studies is dependent on the conclusion on the ED properties with regard to humans and mammals as non-target organisms. If gibberellins GA4/GA7 are identified as ED for humans, new studies on fish and amphibians do not need to be performed in order to avoid unnecessary vertebrate testing.

[5] Commission Regulation (EU) 2018/605 of 19 April 2018 amending Annex II to Regulation (EC) No 1107/2009 by setting out scientific criteria for the determination of endocrine disrupting properties. OJ L 101, 20.4.2018, p. 33–36.

In contrast to the RMS the applicant (European Gibberellin Task Force) is of the opinion that the available evidence is sufficient to conclude that gibberellins GA4/GA7 are not an endocrine disruptor. Below (in *italic*) is the applicant's justification for concluding that gibberellins GA4/GA7 are not an endocrine disruptor:

There is a guideline requirement to consider the potential for the active substance gibberellins (GA4/7) to provoke responses caused by ED in non-target organisms according to newly published criteria. Consideration of the available information is provided below in accordance with recently published guidance from ECHA & EFSA (2018)[6] *and with reference to appropriate additional information available in the recently updated OECD Guidance Document 150*[7]*.*

As a naturally-occurring and ubiquitous substance in plant tissues and other biological matrices, GA4/7 is ever-present in the diets of organisms above the primary producer trophic level. Consequently as herbivorous and omnivorous consumer organisms have co-evolved with their food sources, they have done so with GA4/7 a constant constituent of their diet. It is therefore unlikely that a naturally-occurring PGR such as GA4/7 elicits adverse responses in (sub)populations of non-target organisms when exposed at PGR concentrations in the same range as those habitually encountered in their normal diets.

Mammals
According to the ECHA & EFSA (2018) guidance, the range of mammalian toxicity studies that provide the human health data set may be used as source of information to gauge the likelihood of ED effects occurring in wild (sub)populations of non-target mammals. If a substance is identified as meeting the criteria from the human health perspective, the ED criteria will generally also be met for non-target mammal populations, provided that the adverse effects on reproduction, growth/development, and other relevant adverse effects are likely to impact on (sub)populations.

The preceding review of the regulatory mammalian toxicology dataset has concluded that the overall weight of evidence (WoE) indicates that there is no convincing evidence for GA4/7 being a potential endocrine disrupter. On this basis, ED effects and adverse impacts on (sub)populations of non-target mammals are not expected to follow from PPP-use of gibberellins.

Birds
According to current joint ECHA & EFSA (2018) guidance avian study types potentially able to provide insight into ED activity are reproductive toxicity studies, but their usefulness is limited because the parameters typically determined support only indications of effects that may be responsive to an ED mechanism, without providing diagnostic confirmation. Modified studies that include measurements of estradiol, testosterone and/or thyroid levels would be required to provide evidence of possible estrogenic (E), androgenic (A), thyroidal (T) or steroidogenic (S) modalities of ED activity, and histopathology examinations in the thyroid and gonads and/or phenotypic and genotypic sex ratio determination would be needed to detect the possible presence of EATS-mediated effects. No such investigations are available for GA4/7.

Avian reproduction studies with GA4/7 were waived on the basis that exposure of birds from the proposed uses of PPP-GA4/7 is not expected to exceed exposure from environmental sources of GA4/7 including naturally occurring GAs in plant tissues, such as vine seeds and grapes and other fruits, leaves and shoots. Consequently, no avian reproduction studies have been performed with GA4/7 and it is not possible to draw a direct conclusion on the ED properties of GA4/7 in birds. However, the overall WoE provided by the regulatory mammalian toxicology dataset indicates that there is no convincing evidence for GA4/7 being a potential endocrine disrupter. Given the points noted above, including the general conservatism of endocrine systems between vertebrate groups (e.g. birds and mammals), ED effects and adverse impacts on (sub)populations of birds are not expected to follow from PPP-use of gibberellins and studies to explore possible ED effects of GA4/7 in birds are considered to be unjustified.

Reptiles
No studies are available to provide insight on potential ED effects of GA4/7 in reptiles and established, validated and relevant test methods for these taxa do not currently exist. According to ECHA & EFSA (2018) guidance, research is needed to establish whether extrapolations to reptiles from ED effects (or absence thereof)

[6] ECHA & EFSA (2018). Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009. EFSA Journal 2018;16(6):5311, 135 pp. https://doi.org/10.2903/j.efsa.2018.5311
[7] OECD (2018). Revised Guidance Document 150 on Standardised Test Guidelines for Evaluating Chemicals for Endocrine Disruption.

in other groups of terrestrial vertebrates are scientifically justified. Nevertheless, the points previously made regarding the ubiquity of exposure to natural sources of gibberellins apply equally to reptiles.

In the absence of relevant studies it is not possible to draw a definitive and direct conclusion on the ED properties of GA4/7 in reptiles. However, given the points noted above and the general conservatism of endocrine systems between vertebrate groups (e.g. birds and mammals), studies to explore possible ED effects of GA4/7 in reptiles are considered to be unjustified.

Amphibians
Regulatory studies developed with amphibians target parameters during late stages of metamorphosis from tadpole to adult that are associated with thyroid activity. Accelerated and asynchronous development (characterised by disruption of relative timing of the morphogenesis or development of different tissues and the inability to establish the developmental stage of an animal clearly by reference to developmental landmarks) are thyroid-mediated effects. However delayed development alone is not necessarily an indicator of anti-thyroid activity, which would require supporting evidence from histopathological examination of thyroid tissue. The recommended regulatory test is the Amphibian Metamorphosis Assay (AMA) published as OECD TG 231.

No amphibian studies of regulatory standard have been performed with GA4/7 and it is therefore not possible to draw a conclusion on the ED properties of GA4/7 in amphibians from direct evidence gained from validated, regulatory-standard tests.

According to OECD GD 150 (§93) cross-species extrapolation is well-established for thyroid activity between amphibians and mammals, based on comparisons of outcomes from screening procedures similar to the AMA and in thyroid-sensitive mammalian screens. Based on consideration of 41 thyroid-active substances[8] identified from peer-reviewed public literature or government agency reports, there was strong agreement between the results for the two groups of vertebrates. Thyroid activity was indicated in amphibians, but not for mammals for only one substance and none of the substances found to be active in mammals were inactive in amphibians. It is concluded - despite considerable variation in the type and degree of effect seen in rats and frogs – that it is possible to make useful predictions of in vivo thyroid activity across the vertebrate spectrum, from amphibians to mammals and vice-versa.

Despite the inability to draw on direct evidence from validated amphibian test designs, an assessment of the likelihood of thyroid-mediated effects may therefore be extrapolated from the corresponding findings of regulatory standard studies of mammalian toxicology. The database of the mammalian toxicology of GA4/7 provides no compelling indication of thyroid activity; indeed the weight of evidence points to the contrary. Given the points noted above and the general conservatism of endocrine systems between vertebrate groups, there is no cause to expect that GA4/7 may interfere with thyroid activity in amphibians. Studies to explore possible ED effects of GA4/7 in amphibians are not considered to be justified.

Fish
*A reliable, guideline-comparable study was performed to determine the toxicity of gibberellic acid (GA3) technical a.s. (91.8%), here serving as a surrogate for GA4/7, to the early life-stages of the fathead minnow ([redacted] **al., 2016; CA 8.2.2.1**). During the 33-day study embryos and hatchlings were exposed to nominal concentrations of 0 (control and DMF solvent control), 0.63, 1.3, 2.5, 5.0 and 10 mg a.s./L (<LOQ, 0.64, 1.3, 2.7, 5.2 and 11 mg a.s./L, mean measured). There were no significant, treatment-related impacts on the hatching success, survival or growth (length and weight) of fish fry compared to the pooled control at any of the exposure concentrations. Growth (determined by wet weight) was not significantly affected compared to the untreated control.*

The study included no markers capable of detecting endocrine disturbances and was not designed for that purpose, however according to OECD GD 150, the endpoints of this test procedure that are related to hatchability and early development may be sensitive to, but are not diagnostic of E, A, T and S-mediated disturbances. ECHA & EFSA (2018) guidance additionally notes that some thyroid system disruptors are reportedly able to interfere with the metamorphosis of fish as they develop from the embryo stage to larvae, causing symptoms that are detectable in fish ELS tests. The referenced (Nelson et al., 2016[9]; Stinckens et al.,

[8] Pickford, D.B. (2010). Screening chemicals for thyroid-disrupting activity: A critical comparison of mammalian and amphibian models. *Critical Reviews in Toxicology*. Vol. 40/10, pp 845-892. https://doi.org/10.3109/10408444.2010.494250
[9] Nelson,K.R. *et al*. (2016). Impaired anterior swimbladder inflation following exposure to the thyroid peroxidase inhibitor 2-mercaptobenzothiazole part I: fathead minnow. *Aquatic Toxicology* **173**, 192-203. https://doi.org/10.1016/j.aquatox.2015.12.024

2016[10]) effects were manifested as impaired swim-bladder function resulting from exposure to the same substance in two species of fish. No evidence of such influence of GA3, or any other growth-related impact suggestive of potential thyroid-mediated ED activity is indicated in the findings of the [redacted] et al. (2016) study. Since the ELS tests are performed with sensitive, early development stages of fish, they provide no insight into possible ED disturbances affecting reproduction at later stages of the life cycle. According to ECHA & EFSA (2018) guidance, 'substances ***that are suspected of causing such effects*** *(emphasis added) should be tested by an appropriate procedure designed to deliver relevant diagnostic endpoints'. None of the available data gives cause to suspect that such effects may have occurred in fish exposed to GA3. Moreover, the database of the mammalian toxicology of GA4/7 provides no clear and consistent indication of potentially ED-related reproductive effects and there is consequently no cause to expect that exposure to GA4/7 may cause such effects in fish. Studies to explore possible ED effects of GA4/7 via E, A, T and S modalities in fish are therefore not warranted.*

Invertebrates and terrestrial arthropods
ECHA & EFSA (2018) guidance provides only limited information relevant to invertebrates and terrestrial arthropods.

According to OECD TG 150, many systems in invertebrates are distinct from those in vertebrates, so the conservation of endocrine systems that permits cross-species extrapolation between vertebrate taxa does not apply from vertebrates to invertebrates or vice-versa. Furthermore, endocrinology in most invertebrates is currently poorly understood and there is a corresponding lack of diagnostic screening endpoints for these taxonomic groups. Some in vitro screens are available to detect juvenile hormone and ecdysteroid activity in arthropods, but none of these have been standardised and validated internationally. An Adverse Outcome Pathway (AOP) has been developed for ecdysone receptor (EcR) agonism resulting in lethal moulting disruption in arthropods (Song et al., 2017)[11]. The chemical domain of this AOP includes both steroidal and non-steroidal ecdysone receptor agonists that are known to interfere with moulting in arthropods. They include ecdysone, 20-hydroxyecdysone, ponasterone A, muristerone A, makisterone A, cyasterone and inokosterone. Non-steroidal agonists include 1,2-dibenzoyl-1-tert-butylhydrazine, tebufenzide, methoxyfenozide, halofenozide and chromafenozide. Known non-steroidal agonists mainly belong to groups of chemicals with similar structures, such as dibenzoylhydrazine, aclaminoketone and tetrahydroquinolone. The authors provide supporting information that lists these and other, similar compounds in a table of experimentally verified EcR agonists in insects and crustaceans. Gibberellins are not included among them.

However, OECD GD 150 (§90) cautions that although a number of invertebrate assays with apical endpoints have been included in the document, these assays rarely provide information on MOA and they may also respond to non EASs. As yet, the OECD has not standardised any mechanistic in vitro assays for MOA which occur in invertebrates. This implies that it may currently be impossible to conclude whether a substance is an ED in these phyla, although non-standardised in-vitro assays are available for some MOA in invertebrates – e.g. ecdysteroid and juvenile hormone activity in arthropods.

*A reliable, guideline- and GLP- compliant study was performed to determine the chronic toxicity of gibberellins (GA4/7) technical a.s. (90.6) to the freshwater crustacean Daphnia magna (**Juckeland, 2014; CA 8.2.5.1**). The reproductive output of the parental generation initially introduced to the test system as neonate juveniles was recorded over the course of the 21-day study. Daphnids were exposed to analytically confirmed nominal concentrations of 0 (control), 0.333, 1.00, 3.00 and 9.00 mg a.s./L. The first juveniles were observed in the control and all GA4/7 treatment groups after 11 days and it may therefore be deduced that exposure to GA4/7 caused no interference with the growth (including a sequence of moults) and maturation of the parental daphnids. One adult mortality occurred in the 0.333 mg/L treatment after 19 days and a second mortality occurred in the 9.00 mg/L exposure group after 2 days. There was consequently no statistically significant or dose-responsive impact on parental survival. Although a statistically significant reduction (9.5%) in juvenile production was recorded at 9.00 mg/L compared to the reproductive performance of the untreated control, there was no significant impact on the overall intrinsic rate of population growth at any of the GA4/7 concentrations tested.*

[10] Stinckens, E. *et al*. (2016). Impaired anterior swimbladder inflation following exposure to the thyroid peroxidase inhibitor 2-mercaptobenzothiazole part II: Zebrafish. *Aquatic Toxicology*, **173**, 204-217. https://doi.org/10.1016/j.aquatox.2015.12.023

[11] Song, Y., Villeneuve, D.L, Toyota, K., Iguchi, T and Tollefsen, K.E. (2017). Ecdysone Receptor Agonism Leading to Lethal Molting Disruption in Arthropods: Review and Adverse Outcome Pathway Development. *Environ. Sci. Technol*. 2017, **51**(8),4142-4157 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6135102/

Thus, whereas some reports found in the public domain literature propose that exposure to GA3, and consequently GA4/7, caused endocrine-mediated adverse impacts on the fecundity of grasshoppers (Neumann Visscher, 1980) or fatal disruption of moulting in locusts (Abdellaoui, 2013), the guideline- and GLP-compliant D. magna reproduction study provides no supporting evidence for these effects.

Discussion & Conclusion

The scientific criteria for determining endocrine disrupting (ED) properties according to Commission Regulation (EU) 2018/605 were finalised and published, with an implementation date of 20 October 2018. Under this amendment to the EU regulation for pesticides a substance shall be considered to have ED properties with a potential to cause adverse effects in non-target organisms if it meets the following criteria unless there is evidence that the observed adversity is not relevant at the (sub)population level:

1. *It shows an adverse effect in non-target organisms that constitutes a change in the morphology, physiology, growth, development, reproduction or life span of an organism, system or (sub)population that results in an impairment of functional capacity, an impairment of the capacity to compensate for additional stress or an increase in susceptibility to other influences;*
2. *It has an endocrine mode of action, i.e. it alters the function(s) of the endocrine system;*
3. *The adverse effect is a consequence of the endocrine mode of action.*

Utilising the available toxicology and ecotoxicology information for GA4/7, the European Gibberellin Task Force II (EGTF, otherwise known as Gibberellin Task Force) has performed an evaluation of the potential for GA4/7 to be an endocrine disruptor according to the "Guidance for the Identification of Endocrine Disruptors in the Context of Regulations (EU) No. 528/2012 and (EC) No. 1107/2009" (EFSA Journal 2018; 16(6): 5311).

With respect to mammalian toxicology, the conservative nature of the ED Excel spreadsheet proforma leads to an outcome in which there are some results for GA4/7 that are suggestive of there being some endocrine mediated changes in the studies performed. However, a detailed assessment indicates that the overall weight of evidence (WoE) rests in favour of the conclusion that GA4/7 does not present as a potential endocrine disrupter.

With respect to potential ecotoxicological effects upon non-target organisms, a review of the mammalian toxicology information does not indicate a potential ED hazard for wild mammals. Relevant evidence for effects in other phyla of concern: birds, reptiles, amphibians, fish and invertebrates is considerably less abundant. However, a review of the extant information for GA4/7 and an acknowledgement of the conservation of EATS endocrine modalities across phyla does not predict an ED hazard to (sub)populations of non-target organisms.

Gibberellins are ubiquitous in higher plants. The natural occurrence of gibberellins was reviewed by Macmillan, 2002. This publication is summarised in MCA Section 6 (CA 6.0; CA 6.10.2/02), with the data demonstrating the widespread occurrence of GAs in 128 plants, 7 fungi and 7 bacteria species. GA4 has been found in 54 plant species, across 29 different families, in seeds, leaves, shoots, buds, fruits and pollen. GA7 has been found in 14 plant species, across 9 different families, including seeds, leaves, shoots and pollen. In addition, both GA4 and GA7 has been found in fungi and bacteria species. GA4 and GA7 have been identified in many plant tissues considered to be (or to develop into) edible tissues. Therefore, dietary exposure to naturally occurring GA4/7 is likely to have occurred for millennia, with no indication of adversity in the human population.

It has been established that reproductive tissues of plants (e.g. anthers, pollens and developing seeds) generally have higher concentrations of GAs than vegetative tissues. Concentrations of up to 10 mg/kg have been reported in endosperm and/or immature cotyledons of some species (Hedden; 2003; reported in EU DAR and addenda; Hungary, 2011). Seeds (legume vegetables, dried pulses, oilseeds and cereals) account for between 1.3 and 53% of total mean food intake (chronic consumption data extracted from PRIMo rev. 3.1 from 30 MS diets and 6 GEMS/Food Cluster diets). Therefore, significant dietary exposure to naturally occurring gibberellins already occurs in current EU diets.

In apples, naturally occurring total gibberellins have been measured at concentrations of up to 0.06 mg/kg. Levels of GA4 were recorded up to 0.012 mg/kg (Zhang et al., 2010; MCA Section 6 CA 6.0 CA 6.10.2/04). Following foliar application of GA4/7 in supervised residue trials conducted on apple and pear, including overdosed trials (2.8 N), all residues of GA4 and GA7 were below the limit of quantification (0.05 mg/kg). Therefore, there is no signicant difference between naturally occuring "residues" of GA4/7 and those derived from the representative agronomic use on pome fruit.

A detailed evaluation of the toxicological and ecotoxicological data and consideration of the available data regarding dietary exposure, leads to the conclusion that there is no robust or reliable evidence for GA4/7 to be considered an endocrine disrupter.

B.9.2.5. Acute toxicity to aquatic invertebrates

B.9.2.5.1. Acute toxicity to Daphnia magna

a) Previous evaluation (2005-2011)

Three acute toxicity studies testing both gibberellins GA4/GA7 (one study) and gibberellic acid GA3 (two studies) to *Daphnia magna* were evaluated as part of the EU review for the inclusion of GA4/GA7 in Annex I and are available in the EU DAR. These studies were considered acceptable in the EFSA conclusion for the risk assessment of aquatic invertebrates. The critical study testing GA4/GA7 with *Daphnia magna* is considered appropriate for the current assessment to support renewal of GA4/GA7 and no new studies are submitted assessing the acute toxicity to *Daphnia*. Full details of the study are provided in the EU DAR and related documents and references are listed in DRAR Vol.2. As stated in the residue definition in DRAR Vol 3 CA B.8.6, there are no surface water metabolites of environmental concern and the data provided are therefore limited to the active substance.

PREVIOUS EVALUATION	This study was evaluated in the original DAR and has been considered by EFSA. No new evaluation has been performed. The conclusion has not been changed.
Data point addressed:	CA 8.2.4.1/01 (II A 8.2.4/01 in original DAR)
Author(s) (year):	Sayers, L.E. (2004b)
Title:	Gibberellins A4 & A7 – Acute toxicity to water fleas (*Daphnia magna*) under static conditions
Laboratory report / project number:	13828.6102
Testing facility:	Springborn Smithers Laboratories, Wareham, USA
Published:	No
Test guideline used:	OECD 202 (1984); Method C.2 of Commission Directive 92/69/EEC
Deviations:	None
GLP:	Yes
EU Agreed Endpoint:	48 h (static) EC_{50} > 100 mg a.s./L (nominal)

Executive summary

The acute toxicity of gibberellins (GA4/GA7) to *Daphnia magna* was determined in a 48-hour test under static conditions. The 48-hour EC_{50} is > 97 mg a.s./L (mean measured), the highest concentration tested, based on observations of immobilisation. The NOEC is considered to be 97 mg a.s./L.

I MATERIALS AND METHODS

MATERIALS

Test material:	Gibberellins A4 + A7 (GA4/A7)
Lot/Batch No.:	107-554-CD
Purity:	90.3%
Stability of test compound:	Adequately stable under the conditions of the study. Samples of test media collected during the test performed under static conditions had mean measured concentrations that ranged from 83% to 197 of nominal concentrations.

Test organism:	*Daphnia magna*
Source:	Test facility in-house stock culture.
Age at test initiation:	Juvenile, less than 24 h old.
Treatments:	Six groups: dilution water control and gibberellins treatments at nominal concentrations of 6.3, 13, 25, 50 and 100 mg/L.
Number of animals/group	20/treatment group, with 5 daphnids per replicate 250 mL glass beaker containing 200 mL medium.
Duration:	48 hours.
Conditions:	The test was performed under static conditions. Measurements of pH, temperature and dissolved oxygen concentrations were made at initiation and after 24 and 48 h in one replicate vessel per treatment and temperature was also recorded continuously in a single vessel. The daphnids were not fed throughout the exposure period and the test media were not aerated.
Temperature:	19 to 21°C.
pH:	7.3 to 7.8.
Dissolved oxygen:	8.1 to 8.8 mg/L (>60% ASV).
Total hardness:	170-180 mg $CaCO_3$/L.
Photoperiod:	16 hours.
Confirmatory analysis:	Samples were collected from each preparation at test initiation and from individual replicate test vessels (pooled according to treatment) at the end of the test and analysed for gibberellins by HPLC/UV. The limit of quantitation of the analytical method was 0.036 mg a.s./L.
Observations:	Checks for immobilisation were made after 24 and 48 hours.
Statistical analysis:	Not required.

II RESULTS AND DISCUSSION

A FINDINGS

Mean measured gibberellins concentrations in samples taken from the test substance treatments ranged from 83% to 97% of nominal. The biological endpoints were expressed in relation to the mean measured concentration of the test substance.

A single immobilised daphnid (5%) was recorded in the control group, but no immobilisation was observed in any of the gibberellins treatment groups throughout the test.

B VALIDITY

The test was considered valid.

III CONCLUSIONS

The acute toxicity of gibberellins (GA4/GA7) to *Daphnia magna* was determined in a 48-hour test under static conditions. The 48-hour EC_{50} is > 97 mg a.s./L (mean measured), the highest concentration tested, based on observations of immobilisation. The NOEC is considered to be 97 mg a.s./L.

RMS comments and conclusion:

The OECD 202 *Daphnia* sp. acute immobilisation test to assess effects of chemicals towards daphnids, by measuring immobilisation at 24 hours and 48 hours and comparing it with control values to determine 48h EC_{50}.

Validity: According to OECD 202 test guideline the Daphnia acute immobilisation test is considered

acceptable if the following validity criteria are met:

- In the control, including the control containing the solubilising agent, not more than 10 per cent of the daphnids should have been immobilised **(in test: 5% or 1 daphnia, condition fulfilled)**

- The dissolved oxygen concentration at the end of the test should be ≥ 3 mg/l in control and test vessels **(in test: 8.1. – 8.8 mg/L, condition fulfilled)**

Mean measured concentrations of GA4/GA7 ranged from 83% to 97% of nominal values. The endpoints are expressed as nominal.

The study report states the pH in this study ranged from 7.3 to 7.9.

The study report does not provide information regarding aeration of test vessels, whether the test animals were first brood progeny or whether test vessels were loosely covered to prevent evaporation. The study report states that no deviations from the protocol occurred during this study. The RMS considers the results of the study to be valid.

Acceptability of the analytical methods used in the test: /

Endpoints: The 48-hour EC_{50} is 100 mg a.s./L (nominal), the NOEC is 100 mg a.s./L (nominal).

Conclusion of the RMS: The *Daphnia* acute immobilisation test is considered valid.

b) Evaluation of additional data for the purpose of renewal of Annex I inclusion

No additional data was submitted or required for the purpose of renewal of approval of gibberellins GA4/GA7.

B.9.2.5.2. Acute toxicity to additional aquatic invertebrate species

Gibberellins GA4/GA7 does not have an insecticidal mode of action and does not show insecticidal activity. Therefore, according to EU Reg. 283/2013 it is not required to test the acute toxicity to additional aquatic invertebrate species. No further data available.

B.9.2.6. Long-term and chronic toxicity to aquatic invertebrates

B.9.2.6.1. Reproductive and developmental toxicity to Daphnia magna

a) Previous evaluation (2005-2011)

Daphnia magna reproduction test was not submitted for the first inclusion of gibberellins GA4/GA7. A data gap was identified in EFSA conclusions (EFSA Journal 2012; 10(1):2502).

b) Evaluation of additional data for the purpose of renewal of Annex I inclusion

No data on the long-term and chronic toxicity to aquatic invertebrates were submitted during the Annex I inclusion of gibberellins GA4/GA7. A new *Daphnia magna* reproductive toxicity test with GA4/GA7 is submitted for the purposes of renewal to address the previous EFSA data gap (EFSA Journal 2012;10(1):2502). Full details of the new study are summarised below (CA 8.2.5.1/01) and references are listed in DRAR Vol.2.

Data point addressed:	CA 8.2.5.1/01
Author(s) (year):	Juckeland, D. (2014)
Title:	Toxicity of gibberellins (GA4/GA7) technical to *Daphnia magna* in a 21-day semi-static reproduction test
Laboratory report / project number:	14 10 48 073 W
Testing facility:	BioChem agrar, Gerichshain, Germany
Published:	No
Test guideline used:	OECD 211 (2012)
Deviations:	None
GLP:	Yes
Endpoint:	21 d NOEC (reproductive output) = 3.00 mg a.s./L (nominal) 21 d EC_{50} (reproductive output) >9.00 mg a.s./L (nominal)

Executive summary

A 21-day test was set up under semi-static conditions to assess the effect of GA4/GA7 technical on the reproductive output of *Daphnia magna.* Five concentrations of test item were tested: 0.111, 0.333, 1.00, 3.00 and 9.00 mg a.s./L based on nominal concentrations. The 21 d NOEC based on reproductive output was determined to be 3.00 mg a.s./L (nominal). The 21 d EC_{50} based on reproductive output was determined to be >9.00 mg a.s./L (nominal).

I MATERIALS AND METHODS

A MATERIALS

Test item:	Gibberellins (GA4/GA7) Technical powder
Lot No.:	20130204
Purity:	90.6 % a.i.
Expiry date:	February 2017
Description:	White powder
Stability of test compound:	Adequately stable under the conditions of the study. Samples of expired test media collected at renewals during the test performed under semi-static conditions had measured concentrations that ranged from 91% to 107% of nominal concentrations.

Test organism:	*Daphnia magna* STRAUS
Source:	Landesanstalt für Umweltschutz Baden-Württemberg, Griesbachstr. 1, 76185 Karlsruhe, Germany. Animals used in the test were held and bred in the test facility under standardised laboratory conditions
Age at test initiation:	<24 hours old and not first brood progeny
Temperature:	19.3 – 20.7 °C
Light intensity:	not exceeding 15-20 $\mu E \cdot m^{-2} \cdot s^{-1}$; measured approximately 24 $\mu E \cdot m^{-2} \cdot s^{-1}$
Photoperiod:	16 h light; 8 h darkness
Dissolved oxygen:	7.43 – 9.16 mg/L
pH:	7.32 – 7.89
Water hardness:	225 – 231 mg/L $CaCO_2$

B STUDY DESIGN

Test vessels comprised a glass beaker containing 50 mL of medium. One parent daphnid was placed in each test vessel, with ten test vessels per test concentration. An additional 3 vessels were set up per concentration for measurement of water quality, analysis and specimen retention. The test medium of Elendt M4 medium was prepared with air saturated deionised water according to OECD 211.

Five concentrations of test item were tested: 0.111, 0.333, 1.00, 3.00 and 9.00 mg a.s./L based on nominal concentrations. The control vessels contained test medium only. The test medium was prepared at least 12 hours before the test start or renewal and aerated overnight in the same cabinet and at the same temperatures as the Daphnia test was performed.

The test medium was renewed three times per week. A second series of test vessels was prepared for this purpose on each occasion and the parent animals were transferred to them by a glass pipette of suitable diameter. The volume of the medium transferred with the Daphnia was minimised.

Daphnids were fed three times per week during medium renewals with living algal cells of *Desmodesmus subspicatus*, added directly to the test vessels at 0.1-0.2 mg/C/daphnid/day.

The offspring produced by each parent animal was removed and counted daily from the appearance of the first brood to prevent them consuming food intended for the adult. At the end of the test, the total number of living offspring produced per parent animal alive at the end of the test was assessed. Mortality among the parent animals was recorded daily, at least at the same times as offspring was counted. In addition, the time to production of the first brood was reported.

The test concentrations were determined at the test start, once a week, and at the test end in the freshly prepared and spent test solutions of the control and of the test item concentrations (mean values).

II RESULTS AND DISCUSSION

A FINDINGS

The measured test concentrations of Gibberellins (GA4/GA7) technical in the test solutions remained within a range of 87 to 104 % of nominal values in the freshly prepared test solutions at the start of the test and at each renewal and within a range of 91 to 107 % of nominal in the spent solutions at each renewal and at the end of the test (after 21 days). Therefore, the results for Gibberellins (GA4/GA7) technical are based on the nominal concentrations.

A summary of the biological results is presented in

the table below.

Table 9.2.6.1/01- 1 : Summary of reproductive output of *Daphnia magna* exposed to Gibberellins (GA4/GA7)

Nominal test concentration (mg a.s./L)	Total no. of living offspring 21 days after application			Reduction in total number of living offspring relative to control (%)[a]	Mortality of parent daphnid (%)	Mean of intrinsic rate of population growth, r	Inhibition of intrinsic rate,r, relative to control (%)[a]
	Total mean	Average per female	Standard deviation				
Control	929	92.9	4.75	-	0.0	0.23	0.0
0.111	933	93.3	7.76	-0.4	0.0	0.23	-0.9
0.333	845	93.9	7.25	-1.1	10.0	0.23	0.7
1.00	924	92.4	9.42	0.5	0.0	0.22	2.1
3.00	882	88.2	13.0	5.1	0.0	0.20	11.0
9.00	757	84.1	8.21	9.5*	10.0	0.19	14.5

[a] Negative values for inhibition demonstrate a higher number of living offspring relative to the control

* Significant difference relative to control (Williams Multiple Sequential t-test; $p \leq 0.05$, one-sided)

The first offspring were observed in all treatment groups after 11 days. At the test concentration of 0.333 mg a.s./L one parent animal died after 19 days and at the test concentration of 9.00 mg a.s./L one parent animal died after 2 days.

A summary of the statistical analysis is presented in the table below.

Table 9.2.6.1/01- 2 : Effects of GA4/GA7 technical: summary of statistical analysis

Effect concentration	Gibberellins GA4/GA7 technical mg a.s./L, nominal		
	Reproductive output (no. of offspring per survivor)	Parent mortality	Intrinsic rate of population growth
21 d NOEC	3.00	≥ 9.00	3.00
21 d LOEC	9.00	> 9.00	9.00
21 d EC_{50}	> 9.00	> 9.00	> 9.00

B VALIDITY

The test was considered valid as the following validity criteria were met:

- Parent mortality in the control at the test end was ≤20 % (0 %)
- Mean number of live offspring produced per surviving parent in the control at the test end was ≥60 % (92.9 %)

III CONCLUSIONS

A 21-day test was set up under semi-static conditions to assess the effect of GA4/GA7 technical on the reproductive output of *Daphnia magna.* Five concentrations of test item were tested: 0.111, 0.333, 1.00, 3.00 and 9.00 mg a.s./L based on nominal concentrations. The 21 d NOEC based on reproductive output was determined to be 3.00 mg a.s./L (nominal). The 21 d EC_{50} based on reproductive output was determined to be > 9.00 mg a.s./L (nominal).

RMS comments and conclusion:

The primary objective of the OECD 211 *Daphnia magna* reproduction test is to assess the effect of chemicals on the reproductive output of *Daphnia magna*.

Validity: According to the OECD 211 test guideline the early-life stage toxicity test is considered acceptable if the following validity criteria are met:

For a test to be valid, the following performance criteria should be met in the control(s):

- the mortality of the parent animals (female *Daphnia*) does not exceed 20% at the end of the test (**in test: 0% in control, 10% in treatment group with 9.00 mg/L, condition fulfilled)**
- the mean number of living offspring produced per parent animal surviving at the end of the test is > 60 (**in test: 92.9, condition fulfilled)**

The RMS found no deviations from OECD 211 test guideline.

Acceptability of the analytical methods used in the test: Method was successfully validated regarding linearity, accuracy, precision, LOQ and selectivity and is therefore accepted for determination of GA4/7 in Elendt M4 medium according to SANCO/3029/99. No further data required. The assessment is reported in Section B.5.1.2.6.

Endpoints: The 21 d NOEC based on reproductive output was determined to be 3.00 mg a.s./L (nominal). The 21 d EC_{50} based on reproductive output was determined to be > 9.00 mg a.s./L (nominal).

Conclusion of the RMS: The *Daphnia magna* reproduction toxicity test is considered valid.

B.9.2.6.1. Reproductive and developmental toxicity to additional aquatic invertebrates species

Gibberellins GA4/GA7 does not have an insecticidal mode of action and is not an insect growth regulator. Therefore, according to EU Reg. 283/2013 it is not required to test the reproductive and development toxicity of additional aquatic invertebrate species. No further studies are available or necessary.

B.9.2.6.2. Development and emergence in Chironomus riparius

Gibberellins GA4/GA7 is not an insect growth regulator. Therefore, according to EU Reg. 283/2013 it is not required to test the development and emergence in *Chironomus riparius.* No further studies are available or necessary.

B.9.2.6.3. Sediment dwelling organisms

The available data for *Daphnia* demonstrate a low chronic toxicity to invertebrates (NOEC = 3.00 mg a.s./L; CA 8.2.5.1/01). No data are available regarding the % of gibberellins GA4/GA7 in sediment; however, a read across from gibberellic acid GA3 can be applied. Gibberellic acid GA3 was shown to degrade quickly in two water/sediment systems (<2% AR after 59 days) (DRAR for GA3 Vol. 3 CA B.8.2.4). As the amount of active substance in sediment phase does not exceed 10% of applied radioactivity in the water sediment study, testing on sediment-dwelling organisms is not considered necessary for gibberellic acid GA3. Based on structural similarities between gibberellins GA4/GA7 and gibberellic acid GA3 a low affinity of GA4/GA7 to move to sediment can be predicted. The decision that testing of GA4/GA7 on sediment-dwelling organisms is not considered necessary can be further supported by a large margin of safety obtained in chronic risk assessment for aquatic invertebrates. Additionally, gibberellins GA4/GA7 is a naturally occuring substance (see CA 8.9/01, and the review of public domain literature on the presence of endogenous gibberellins in higher plants, algae, mosses and lichens as well as the biosynthesis of gibberellins by soil bacteria and fungi included at Vol.3 CA B.8) and it is therefore assumed that sediment dwelling organisms would be exposed to GA4/GA7 in their natural environment and thus adjusted to it. Gibberellins GA4/GA7 functions as a plant growth regulator and is therefore not expected to have highly toxic effects on insects. As stated in the residue definition in DRAR Vol 3

CA B.8.6, there are no metabolites of environmental concern in sediment. No further studies are available or necessary.

B.9.2.7. Effects on algal growth

B.9.2.7.1. Effects on growth of green algae

a) Previous evaluation (2005-2011)

Two algal growth studies with *Pseudokirchneriella subcapitata* (one with gibberellins GA4/GA7 and one with gibberellic acid GA3) were evaluated as part of the EU review for the inclusion of GA4/GA7 in Annex I and are available in the EU DAR. These studies were considered acceptable in the EFSA conclusion for the risk assessment of effects on algal growth. The study on GA4/GA7 is considered appropriate for the current assessment to support renewal of GA4/GA7 and no new studies are submitted assessing the effects on growth of green alga. The previous study was conducted according to OECD guideline 201 (1984), which has since been updated (2006 and 2011) to include new validity criteria and calculation of EC_x values. Therefore, in addition to the original study summary in the DAR (II A 8.2.6/01; CA 8.2.6.1/01a), an updated summary is submitted below (CA 8.2.6.1/01b) to address the considerations of the updated guideline. Full details of the study are provided in the EU DAR and related documents and references are listed in DRAR Vol.2. As stated in the residue definition in DRAR Vol 3 CA B.8.6, there are no surface water metabolites of environmental concern and the data provided are therefore limited to the active substance.

PREVIOUS EVALUATION	This study was evaluated in the original DAR and has been considered by EFSA. However, an updated OECD guideline is now noted, which requires consideration of updated validity criteria and calculation of EC_x values. These considerations are addressed in CA 8.2.6.1/01b.
Data point addressed:	CA 8.2.6.1/01a (II A 8.2.6/01 in original DAR)
Author(s) (year):	Gries, T. (2000)
Title:	Gibberellins A4 + A7 – Static toxicity test with the freshwater algae *Pseudokirchneriella subcapitata*
Laboratory report / project number:	1042.004.430
Testing facility:	Springborn Laboratories (Europe) AG, Horn, Switzerland
Published:	No
Test guideline used:	OECD 201 (1984); Method C.3 of Commission Directive 92/69/EEC
Deviations:	Changes in pH between the start and end of the test were +1.81, +1.95 and -2.24 units in the control, solvent control and Gibberellins (GA4/GA7) treatments, respectively and all exceeded the normal fluctuation range of ±1.0 unit indicated in the OECD guideline. Nevertheless, cell densities increased by more than x16 within 72 hours in all three treatments, demonstrating that growth was not impaired. Changes in pH are therefore considered not to have affected the outcome of the study.
GLP:	Yes
EU Agreed Endpoint:	96 h (static) E_bC_{50} >100 mg a.s./L (nominal) 96 h (static) E_rC_{50} >100 mg a.s./L (nominal)

Executive summary

The effects of gibberellins (GA4/GA7) on the growth of the unicellular green alga *Pseudokirchneriella subcapitata* was determined in a 96-hour test under static conditions. The 72-hour E_rC_{50} is > 100 mg a.s./L (nominal initial), the limit test concentration, based on impact on specific growth rate. The NOEC is considered to be 100 mg a.s./L.

I MATERIALS AND METHODS

MATERIALS

Test material:	Gibberellin A4 + A7
Lot/Batch No.:	33-263-CD-00
Purity:	65.8% w/w GA4 + 25.0% w/w GA7
Stability of test compound:	Adequately stable under the conditions of the study. Measured concentrations at the end of the 96-hour static test ranged from 71.3% to 97.9% of the nominal limit concentration (100 mg/L).

Test organism:	*Pseudokirchneriella subcapitata*.
Source:	Stock culture maintained at the test facility, originally established with algae obtained from the Plant Physiological Institute, Göttingen, Germany.
Treatments:	Three groups: OECD medium (containing 50 mg $NaHCO_3$/L) control, solvent control containing 100 µL dimethylformamide/L and a single limit gibberellins treatment at a nominal concentration of 100 mg GA4/GA7/L.
Replication	Eight 250 mL glass Erlenmeyer flasks/treatment group, containing 50 mL test medium inoculated with an initial density of 10^4 algal cells/mL and each closed with a stainless-steel cap.
Duration:	96 hours.
Conditions:	The test was performed under static conditions, with the test vessels placed on an orbital shaker in a water bath. Measurements of pH were made at test initiation and termination. Temperatures were recorded continuously.
Temperature:	22.7 to 24.9°C.
pH:	Control: 8.00 at 0 h; 9.81 at 96 h. Solvent control: 7.91 at 0 h; 9.86 at 96 h. GA4/GA7 treatment: 7.92 at 0 h; 5.68 at 96 h.
Photoperiod:	Continuous light (7200 to 7800 lux).
Agitation:	Continuous at 60 rpm.
Confirmatory analysis:	Samples collected from each test concentration at 0 and 96 hours (replicates pooled according to treatment) were centrifuged and analysed for gibberellic acid by HPLC/DAD. The limit of quantitation of the analytical method was 7.62 mg/L and 2.99 mg/L for GA4 and GA7, respectively.
Observations:	Samples of media were taken from each replicate at 0, 24, 48, 72 and 96 hours to determine algal cell densities directly, using a microscope and haemocytometer.
Statistical analysis:	Normality and homogeneity of variances were determined with Shapiro-Wilk's test and Bartlett's test, respectively. The NOEC was determined using Dunnett's test.

II RESULTS AND DISCUSSION

A FINDINGS

Measured GA4 and GA7 concentrations in samples taken from the test substance treatment at test initiation ranged from 102.5% to 104.6% of nominals and were reduced to 97.9% and 71.3% of nominals, respectively, after 96 hours. Biological effect endpoints were therefore related to the nominal initial test substance concentration.

Measurements of algal cell densities are shown in the following table.

Table 9.2.7.1/01a- 1 : Effects on the growth of *P. subcapitata* following 96-hour exposure to gibberellins (GA4/GA7) under static conditions

Nominal gibberellins concentration	Mean[a] cell density (cells/mL × 10^4)					Growth inhibition (%) relative to control after 96 hours	
	0 h	24 h	48 h	72 h	96 h	growth rate	AUGC
0 (control)	1.0	3.7	14.6	72.2	181.5	-2.5	-11
0 (solvent control)	1.0	3.0	14.7	69.1	160.2	n.a.	n.a.
100	1.0	3.4	18.3	80.2	173.4	-1.5	-14

[a] Based on 8 replicates/treatment.

B VALIDITY

The cell density of the solvent control cultures increased by ×69 in 72 h and exceeded the requirement for a minimum 16-fold increase in that time. The test was considered valid at the time of the previous evaluation. A re-evaluation against updated and extended OECD validity criteria is presented below in CA 8.2.6.1/01b.

III CONCLUSIONS

The effects of gibberellins (GA4/GA7) on the growth of the unicellular green alga *Pseudokirchneriella subcapitata* was determined in a 96-hour test under static conditions. The 72-hour E_rC_{50} is > 100 mg a.s./L (nominal initial), the limit test concentration, based on impact on specific growth rate. The NOEC is considered to be 100 mg a.s./L.

RMS comments and conclusion:

Comments for CA 8.2.6.1/01a are given after the summary for CA 8.2.6.1/01b, because both summaries relate to the same study.

PREVIOUS EVALUATION	The original study (CA 8.2.6.1/01a) was evaluated in the original DAR and has been considered by EFSA. However, an updated OECD guideline is now noted, which requires consideration of updated validity criteria and calculation of EC_x values. These considerations are addressed here in CA 8.2.6.1/01b.
Data point addressed:	CA 8.2.6.1/01b
Author(s) (year):	Collison, E. (2017)
Title:	Re-assessment of validity of Gries (2000): Gibberellins A4 + A7 – Static toxicity test with the freshwater algae *Pseudokirchneriella subcapitata*; Report No. 1042.004.430. Springborn Laboratories (Europe) AG, Horn, Switzerland [KCA 8.2.6.1/01]
Laboratory report / project number:	07-048-01/01
Testing facility:	N/A
Published:	No
Test guideline used:	OECD 201 (updated 2011)
Deviations:	N/A
GLP:	N/A
Endpoint:	72 h (static) E_rC_{10} >100 mg a.s./L 72 h (static) E_rC_{20} >100 mg a.s./L 72 h (static) E_rC_{50} >100 mg a.s./L

Executive summary

The study tested only a single concentration of test item (GA4/GA7), 100 mg a.s./L, at which no adverse effects on algal growth were observed. Therefore, no formal calculation of ECx values is necessary and the EC_{10}, EC_{20}

and EC_{50} values were all determined to be >100 mg a.s./L. The NOEC was determined to be ≥100 mg a.s./L. Calculations of the validity criteria according to OECD 201 (updated 2011) were performed and all validity criteria were met. The exponential growth factor in the control and solvent control cultures was at least 16-fold (72.2 and 69.1, respectively), the mean coefficient of variation for section-by-section specific growth rates in the control and solvent control cultures was <35% (19.2% and 23.2%, respectively) and the coefficient of variation for average specific growth rate during the whole test period in the control and solvent control cultures was <7% (6.3% and 5.4%, respectively).

I MATERIALS AND METHODS

ECx calculations

The study tested only a single concentration of test item, 100 mg a.s,/L, at which no adverse effects on algal growth were observed. Therefore, no formal calculation of ECx values is necessary and the EC_{10}, EC_{20} and EC_{50} values were all determined to be >100 mg a.s./L. The NOEC was determined to be ≥100 mg a.s./L.

Validity criteria

The OECD 201 (updated 2011) guideline specifies a number of validity criteria. To calculate whether the criteria were met, calculations were performed in Microsoft Excel using the raw data available in the original study report.

II RESULTS AND DISCUSSION

Results of the relevant validity criteria calculations for the OECD medium control and solvent (DMF) control are presented in the tables below.

Table 9.2.7.1/01b- 1 : Growth increase factor calculations in the control cultures for KCA 8.2.6.1/01

Initial cell count (x 10^4 cells/ml)	1.0
End of study (72h) cell count (x 10^4 cells/ml)	72.2
Exponential growth factor	**72.2**

Table 9.2.7.1/01b- 2 : Growth increase factor calculations in the solvent control cultures for KCA 8.2.6.1/01

Initial cell count (x 10^4 cells/ml)	1.0
End of study cell count (x 10^4 cells/ml)	69.1
Exponential growth factor	**69.1**

The validity criterion of at least a 16-fold exponential growth factor is met.

Table 9.2.7.1/01b- 3 : Coefficient of variation calculations for section-by-section specific growth rates in the control cultures for KCA 8.2.6.1/01

Control Replicate	Daily growth rate*			Mean	Standard deviation	Coefficient of variation %
	0-24h	24-48h	48-72h			
A	1.57	1.20	1.70	1.49	0.26	17.35
B	1.50	1.12	1.91	1.51	0.39	26.06
C	1.19	1.43	1.71	1.44	0.26	17.76
D	1.34	1.53	1.56	1.47	0.12	8.30
E	1.19	1.47	1.17	1.28	0.17	12.94
F	1.46	0.89	1.63	1.33	0.39	29.16
G	1.10	1.85	1.47	1.47	0.37	25.40
H	1.10	1.37	1.54	1.34	0.22	16.64
Mean coefficient of variation of the section by section specific growth rate						**19.20**

* calculated according to point 48, OECD 201 (rev.2011)

Table 9.2.7.1/01b- 4 : Coefficient of variation calculations for section-by-section specific growth rates in the solvent control cultures for KCA 8.2.6.1/01

Solvent Control Replicate	Daily growth rate*			Mean	Standard deviation	Coefficient of variation %
	0-24h	24-48h	48-72h			
A	1.25	1.71	1.61	1.52	0.24	15.69
B	0.92	1.84	1.37	1.38	0.46	33.67
C	1.10	1.72	1.61	1.48	0.33	22.49
D	0.92	1.65	1.35	1.31	0.37	28.21
E	1.34	1.23	1.53	1.37	0.15	11.32
F	0.69	1.70	1.59	1.33	0.55	41.65
G	1.10	1.61	1.69	1.46	0.32	21.81
H	1.25	1.37	1.56	1.39	0.15	11.01
Mean coefficient of variation of the section by section specific growth rate						**23.23**

* calculated according to point 48, OECD 201 (rev.2011)

The mean coefficient of variation for section-by-section specific growth rates in the control and solvent control cultures is within the validity criterion of <35%.

Table 9.2.7.1/01b- 5 : Coefficient of variation calculations for average specific growth rates during the whole test period in the control cultures for KCA 8.2.6.1/01

Control Replicate	Average specific growth rate (0-72h)
A	1.49
B	1.51
C	1.44
D	1.47
E	1.28
F	1.33
G	1.47
H	1.34
Mean	1.42
Standard deviation	0.09
Coefficient of variation %	**6.30**

Table 9.2.7.1/01b- 6 : Coefficient of variation calculations for average specific growth rates during the whole test period in the solvent control cultures for KCA 8.2.6.1/01

Solvent Control Replicate	Average specific growth rate (0-72h)
A	1.52
B	1.38
C	1.48
D	1.31
E	1.37
F	1.33
G	1.46
H	1.39
Mean	**1.40**
Standard deviation	**0.08**
Coefficient of variation %	**5.41**

The coefficient of variation for average specific growth rate during the whole test period in the control and solvent control cultures is within the validity criterion of <7%.

III. CONCLUSIONS

A study of the effects of Gibberellins A4 + A7 on the growth of the unicellular green alga *Pseudokirchneriella subcapitata* [**KCA 8.2.6.1/01a**] was performed in 2000 according to the then current 1984 version of OECD TG 201. The findings of the study have been re-assessed in compliance with the current (adopted 2011) version of OECD TG 201.

The E_rC_{10}, E_rC_{20} and E_rC_{50} values were determined to be >100 mg a.s./L. All validity criteria in accordance with the updated OECD 201 guideline (2011) were met.

RMS comments and conclusion (for CA 8.2.6.1/01a and CA 8.2.6.1/01b):

The purpose of OECD 201 Freshwater alga and cyanobacteria growth inhibition test is to determine the effects of a substance on the growth of freshwater microalgae and/or cyanobacteria.

Validity: According to the OECD 201 test guideline the alga growth inhibition test is considered acceptable if the following validity criteria are met:

For the test to be valid, the following performance criteria should be met:

- The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period. This corresponds to a specific growth rate of 0.92 day-1. For the most frequently used species the growth rate is usually substantially higher **(in test: cell density of the solvent control cultures increased by factor 69, condition fulfilled)**

- The mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2 and 2-3, for 72-hour tests) in the control cultures must not exceed 35%. This criterion applies to the mean value of coefficients of variation calculated for replicate control cultures (**in test: for solvent control cultures – 23.23%, condition fulfilled)**

- The coefficient of variation of average specific growth rates during the whole test period in replicate control cultures must not exceed 7% in tests with *Pseudokirchneriella subcapitata* and *Desmodesmus subspicatus*. For other less frequently tested species, the value should not exceed 10% **(in test: for solvent control cultures – 5.41%, condition fulfilled)**

In this study the Erlenmeyer flasks were closed with stainless steel caps, however the test guideline requires air-permeable stoppers. The RMS considers this to be a minor deviation that does not affect the validity of the results.

The temperature in this study was in the range 22.7°C – 24.9°C; but the test guideline requires the temperature to be in the range 21°C – 24°C. The RMS considers this to be a minor deviation that does not affect the validity of the results.

The pH of control medium increased by 1.95 during the test, which is more than 1.5 as recommended by test guideline. The growth of algae in the study was normal and the study meets validity criteria. This indicates that that growth of algae was not impaired despite the increase in pH. The RMS considers the results of the study to be reliable.

Acceptability of the analytical methods used in the test: /

Endpoints: The E_rC_{10}, E_rC_{20} and E_rC_{50} are > 100 mg a.s./L (nominal).

Conclusion of the RMS: The freshwater alga and cyanobacteria growth inhibition test is considered valid.

b) Evaluation of additional data for the purpose of renewal of Annex I inclusion

No additional data was submitted or required for the purpose of renewal of approval of gibberellins GA4/GA7.

B.9.2.7.2. Effects on growth of an additional algal species

a) Previous evaluation (2005-2011)

Growth inhibition test of additional algal species was not submitted for the first inclusion of gibberellins GA4/GA7. The active substance is not an herbicide; therefore, this study is not a data requirement.

b) Evaluation of additional data for the purpose of renewal of Annex I inclusion

No data on the effects of gibberellins GA4/GA7 on an additional algal species were submitted during the Annex I inclusion of GA4/GA7. A new study testing effects of GA4/GA7 technical on *Navicula pelliculosa* is submitted for the purposes of renewal. Details of the new study are summarised below (CA 8.2.6.2/01) and references are listed in DRAR Vol.3 CA B.9.

Data point addressed:	CA 8.2.6.2/01
Author(s) (year):	Mantilacci, S. (2017)
Title:	Toxicity evaluation of test item Gibberellins GA4/GA7 Technical on *Navicula pelliculosa* in a growth inhibition limit test and validation of the analytical method
Laboratory report / project number:	BT264/17
Testing facility:	BioTecnologie B.T. Srl, Italy
Published:	No
Test guideline used:	OECD 201 (2011)
Deviations:	None
GLP:	Yes
Endpoint:	72 h E_rC_{50} >100 mg test item/L, equivalent to >91.35 mg GA4/GA7/L (nominal)

Executive summary

An algal growth inhibition test was conducted to determine the effect of the test item Gibberellins GA4/GA7 technical on growth of *Navicula pelliculosa*. The test was conducted as a limit test under static conditions, at a nominal concentration of 100 mg test item/L, equivalent to 91.35 mg GA4/GA7/L, for 72 hours. Measured concentrations of GA4 and GA7 throughout the study period were within ±20% of nominals and the biological results were therefore based on nominal concentrations. No significant inhibitory effects of the test item were observed and the E_rC_{50} was therefore determined to be >91.35 mg GA4/GA7/L.

I MATERIALS AND METHODS

A MATERIALS

Test item:	Gibberellins GA4/GA7 Technical
Lot No.:	F0170901
Purity:	91.35% a.i. (w/w)
CAS No.:	GA4 [468-44-0] GA7 [510-75-8] GA4/A7 mixture [8030-53-3]
Expiry date:	September 2019
Description:	White powder
Stability of test compound:	Adequately stable under the conditions of the study. Measured concentrations of GA4 and GA7 at the end of the 72-hour static test were within ±20% of the nominal limit concentration.

Test organism:	*Navicula pelliculosa*
Source:	SAG Culture collection of algae (Göttingen)
Test medium:	EPA/Si growth medium fortified with silicate
pH:	7.5 ± 0.1

Test conditions

Temperature:	21.6-23.6 °C
Light intensity:	5997 -6906 lux
Photoperiod:	Continuous light
pH:	Control group: 7.58 – 8.04; Treated group: 6.55 – 7.29

B STUDY DESIGN

The study was performed for 72 hours in a static system as a limit test, with one control group and one test item concentration (100 mg test item/L, equivalent to 91.35 mg GA4/GA7/L). Each group included six replicates. At the start of the test 100.0 mg of the test item was weighed in a 1000 mL graduated flask and brought up to volume with EPA medium, to obtain a stock solution at 100 mg a.s./L. The solution was sonicated for 20 minutes and maintained on a magnetic stirrer for 4 hours.

100 mL of the test solution was used for each treated replicate. The control group used 100 mL of EPA medium. Each flask was inoculated with an aliquot of algal culture in order to give an initial density of 10^4 cells/mL. During the test, the flasks were placed on a shaker. The algal biomass in each flask and the appearance of the algae were assessed daily by microscope direct count for 72 hours.

For analytical determination of test concentrations (for the two analytes GA4 and GA7), samples were collected from the treated and control groups at the start of the test from fresh test solutions (T0), after 24 hours from the exposed solutions (T24) and at the end of the test from the aged test solutions (T72). At each time point, one control and one test item sample were collected.

II RESULTS AND DISCUSSION

A FINDINGS

A summary of the analytical results is presented in the table below.

Table 9.2.7.2/01- 1 : Summary of analytical results for measured concentrations of GA4 and GA7 in test samples

Sample	Nominal concentration (mg/L)	Mean measured concentration (mg/L)	Mean recovery (%)	Mean measured concentration (mg/L) for treatment group	Mean recovery (%) for treatment group
GA4					
Control (0, 24 and 72 h)	0.00	Not determined			
0 h fresh sample	90.30	100.59	111.40	97.91	108.43
24 h spent sample		97.04	107.46		
72 h spent sample		96.10	106.42		
GA7					
Control (0, 24 and 72 h)	0.00	Not determined			
0 h fresh sample	1.05	1.12	106.28	1.03	98.35
24 h spent sample		1.05	99.78		
72 h spent sample		0.93	88.99		

Measured concentrations of GA4 and GA7 throughout the study period were within ±20% of nominals and the biological results were therefore based on nominal concentrations.

A summary of the mean algal biomass (cell density) in each treatment group is presented in the table below.

Table 9.2.7.2/01- 2 : Summary of algal cell density of *Navicula* following exposure to GA4/GA7

Nominal test item concentration (mg/L)	Cell density (x10^4 cells/mL)			
	0 hours	**24 hours**	**48 hours**	**72 hours**
Control	1.00	2.36	6.47	27.86
100	1.00	2.25	6.19	25.94

A summary of the section-by-section and overall growth rates and percentage inhibition in growth rate relative to the untreated control is presented in the table below.

Table 9.2.7.2/01- 3 : Summary of growth rates of *N. pelliculosa* following exposure to GA4/GA7

Nominal test item concentration (mg/L)	Mean section-by-section growth rate µ			Mean specific growth rate µ (S.D.)	% inhibition in growth rate relative to the control
	0-24 hours	**24-48 hours**	**48-72 hours**	**0-72 hours**	
Control	0.840	1.015	1.462	1.106 (0.034)	-
100	0.795	1.012	1.443	1.083 (0.015)	2.08

S.D. = standard deviation

A summary of the section-by-section and overall yield values and percentage inhibition in yield relative to the untreated control is presented in the table below.

Table 9.2.7.2/01- 4 : Summary of yield values of *N. pelliculosa* following exposure to GA4/GA7

Nominal test item concentration (mg/L)	**Mean section-by-section yield y ($x10^4$ cells/mL)**			**Mean yield value y (S.D.)**	**% inhibition in yield relative to the control**
	0-24 hours	**24-48 hours**	**48-72 hours**	**0-72 hours**	
Control	1.36	4.11	21.39	26.86 (2.66)	-
100	1.25	3.94	19.75	24.94 (1.22)	7.15

S.D. = standard deviation

No significant inhibitory effects of the test item were observed and the E_rC_{50} was therefore determined to be >100 mg test item/L (equivalent to >91.35 mg GA4/GA7/L), the highest concentration tested.

B VALIDITY

The test was considered valid as the following validity criteria were met:

- Biomass in the control cultures increased exponentially by a factor of at least 16 within the 72-hour test period (corresponding to a specific growth rate of at least 0.92 day^{-1}). The observed increase was a factor of 27.9 (mean specific growth rate of 1.108 day^{-1}).
- The mean coefficient of variation for section-by-section specific growth rates in the control cultures did not exceed 35% (observed value = 29.9%).
- The coefficient of variation of average specific growth rates during the whole test period in replicate control cultures did not exceed 10% (observed value = 3%).

III CONCLUSIONS

An algal growth inhibition test was conducted to determine the effect of the test item Gibberellins GA4/GA7 technical on growth of *Navicula pelliculosa.* Measured concentrations of GA4 and GA7 throughout the study period were within ±20% of nominals and the biological results were therefore based on nominal concentrations. No significant inhibitory effects of the test item were observed in the limit test and the E_rC_{50} was therefore determined to be >100 mg test item/L (equivalent to >91.35 mg GA4/GA7/L).

RMS comments and conclusion:

The purpose of OECD 201 Freshwater alga and cyanobacteria growth inhibition test is to determine the effects of a substance on the growth of freshwater microalgae and/or cyanobacteria.

Validity: According to the OECD 201 test guideline the alga growth inhibition test is considered acceptable if the following validity criteria are met:

For the test to be valid, the following performance criteria should be met:

- The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period. This corresponds to a specific growth rate of 0.92 day-1. For the most frequently used species the growth rate is usually substantially higher **(in test: cell density of the solvent control cultures increased by factor 27.9, condition fulfilled)**

- The mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2 and 2-3, for 72-hour tests) in the control cultures must not exceed 35%. This criterion applies to the mean value of coefficients of variation calculated for replicate control cultures (**in test: 29.9%, condition**

fulfilled)

- The coefficient of variation of average specific growth rates during the whole test period in replicate control cultures must not exceed 7% in tests with *Pseudokirchneriella subcapitata* and *Desmodesmus subspicatus*. For other less frequently tested species, the value should not exceed 10% **(in test: 3%, condition fulfilled)**

The study was designed as limit test, therefore only one test concentration was used (100 mg/L). The RMS considers this to be acceptable.

The test guideline requires the number of control replicates to be at least 3; ideally it should be twice the number of replicates used for each test concentration. In this study the number of control replicates is 6, as is number of replicates for the test concentration. The RMS considers this to be acceptable.

Acceptability of the analytical methods used in the test: The analytical method for the determination of GA4/7 in EPA medium is considered valid and acceptable according to SANCO/3029/99 rev.4. No further data required. The assessment is provided in Section B.5.1.2.6.

Endpoints: The E_rC_{10}, E_rC_{20} and E_rC_{50} are > 91.35 mg a.s./L (nominal).

Conclusion of the RMS: The freshwater alga and cyanobacteria growth inhibition test is considered valid.

B.9.2.8. Effects on aquatic macrophytes

a) Previous evaluation (2005-2011)

Growth inhibition test on aquatic macrophytes was not submitted for the first inclusion of gibberellins GA4/GA7. A data gap was identified in EFSA conclusions (EFSA Journal 2012; 10(1):2502).

b) Evaluation of additional data for the purpose of renewal of Annex I inclusion

No data on the effects of gibberellins GA4/GA7 on aquatic macrophytes were submitted during the Annex I inclusion of GA4/GA7. Two new studies testing effects of the representative product, Novagib, on *Lemna minor* and *Myriophyllum spicatum* are submitted for the purposes of renewal to address the previous EFSA data gap (EFSA Journal 2012;10(1):2502). No data on the technical active substance are submitted, but the available formulation studies are considered sufficient to address the data requirements for the active substance as all other components of the formulation Novagib are inert and of no ecotoxicological relevance. Full details of the new studies are summarised in DRAR Vol.3 Novagib B.9.3 (CP 10.2.1/04 and CP 10.2.1/05) and references are listed in DRAR Vol.2.

B.9.2.9. Further testing on aquatic organisms

No further data provided.

B.9.3. EFFECTS ON ARTHROPODS

B.9.3.1. Effects on bees

B.9.3.1.1. Acute oral toxicity to bees

a) Previous evaluation (2005-2011)

An acute oral toxicity study with gibberellins GA4/GA7 was submitted and evaluated as part of the EU review for the inclusion of GA4/GA7 in Annex I and is available in the EU DAR. This study was considered acceptable in the EFSA conclusion for the acute oral risk assessment of bees. The study is considered appropriate for the current assessment to support renewal of GA4/GA7 and no new studies are submitted assessing the acute oral toxicity to bees. Full details of the study are provided in the EU DAR and related documents and references are listed in DRAR Vol.2.

PREVIOUS EVALUATION	This study was evaluated in the original DAR and has been considered by EFSA. No new evaluation has been performed. The conclusion has not been changed.
Data point addressed:	CA 8.3.1.1/01 (II A 8.3.1.1/01 in original DAR)
Author(s) (year):	Hoberg, J.R. (2004a)
Title:	Gibberellins A4 & A7 – Acute oral toxicity test with the honey bee (*Apis mellifera*)
Laboratory report / project number:	13828.6103
Testing facility:	Springborn Smithers Laboratories, Wareham, USA
Published:	No
Test guideline used:	OECD 213; EPPO 170 (1992)
Deviations:	None
GLP:	Yes
EU Agreed Endpoint:	48 h acute oral LD_{50} > 87 µg/bee

Executive summary

The acute oral toxicity of gibberellins (GA4/GA7) to the honey bee *Apis mellifera* was determined in a 48-hour test. The 48-hour contact LD_{50} is > 87 µg a.s./bee (nominal), the highest dose tested. The NOEL is considered to be 87 µg a.s./bee.

I MATERIALS AND METHODS

MATERIALS

Test material:	Gibberellins A4 + A7 (GA4/A7)
Lot/Batch No.:	107-554-CD
Purity:	90.3%
Stability of test compound:	Not reported, but may be considered to be adequately stable under the conditions of this study based on the behaviour observed in other tests.

Test organism:	*Apis mellifera*, Carniolan strain.
Age	Young adult workers taken from the brood frames of a mature hive.
Source:	Colonies maintained in hives at the test facility.
Treatments:	Seven groups: control and acetone solvent control and gibberellins treatments applied at nominal doses of 6.3, 13, 25, 50 and 100 µg/bee. In addition, there were three reference toxicant groups with dimethoate dosed at 0.090, 0.18 ad 0.36 µg/bee.
Replication	Three chambers constructed of polyester mesh attached to a PVC frame (13 ×13 ×13 cm) per treatment group.
Number of bees per group:	30 (10 bees per replicate).
Duration:	48 hours.
Conditions:	The test units were maintained in an incubator. The bees were fed from glass troughs, initially weighed with their contents, and inserted into the test chambers. The troughs initially contained 200 µL of a 50% aqueous sucrose solution with no other addition (control) or amended with 10 µL acetone (solvent control) or a series of stock solutions of gibberellins GA4/GA7 dissolved in acetone.
Temperature:	25 to 26°C.
Relative humidity:	51% to 62%.
Photoperiod:	Continuous near-darkness.
Confirmatory analysis:	None.
Observations:	Bees were observed 4, 24 and 48 hours after dose application to count the numbers of mortalities and individuals exhibiting symptoms of toxicity or abnormal behaviour. A single observation at 24 h was made in the reference toxicant group.
Statistical analysis:	Not required.

II RESULTS AND DISCUSSION

A FINDINGS

Mortality in the control and solvent control groups was 0% after 48 h. There were no mortalities in any of the GA4/GA7 treatment groups and all surviving bees appeared normal in behaviour throughout the study. Surviving bees of the dimethoate reference groups displayed lethargy and/or spasmodic movements at the 24-h observation.

Based on the weights of the feed containers at the start and end of the dose uptake phase, dose consumption was 108, 105, 102, 94, 97, 95 and 87% of the amounts offered in the control, solvent control and the 6.3, 13, 25, 50 and 100 µg gibberellins GA4/GA7 treatment groups, respectively. The maximum consumed dose was therefore 87 µg a.s./bee.

B VALIDITY

Mortality in the control and solvent control groups was below 10%.

After 24 h, mortality in the three dimethoate dose groups ranged from 37% to 97%, indicating that the LD_{50} lay within the 0.10 to 0.35 µg/bee range stipulated by the test guideline.

III CONCLUSIONS

The acute oral toxicity of gibberellins (GA4/GA7) to the honey bee *Apis mellifera* was determined in a 48-hour test. The 48-hour contact LD_{50} is > 87 µg a.s./bee (nominal), the highest dose tested. The NOEL is considered to be 87 µg a.s./bee.

RMS comments and conclusion:

The study has been evaluated according to the OECD 213 Honeybee acute oral toxicity test. The OECD 213 toxicity test is designed to assess the acute contact toxicity of pesticides and other chemicals to adult worker honeybees.

Validity: According to the OECD 213 test guideline the honeybee acute contact toxicity test is considered acceptable if the following validity criteria are met:

- the average mortality for the total number of controls must not exceed 10 per cent at the end of the test **(in test: 0%, condition fulfilled)**

- the LD_{50} of the toxic standard meets the specified range 0.1 – 0.35 µg/bee **(in test: mortality in 0.09 µg/bee dimethoate group was 37%, mortality in 0.36 µg/bee dimethoate group was 97%, condition fulfilled)**

The concentration of solvent acetone in final treatment solution was 5%, which is more than 1% as recommended by the test guideline. The concentration of acetone was equal in all treatment solutions and a solvent control group was used in the study. As there were no mortalities in control, solvent control or GA4/GA7 treatment groups, the RMS considers the results to be valid.

Stability of the test compound was not tested in this study. The RMS agrees with the applicant that the test compound can be considered adequately stable based on the results of other studies submitted for re-registration.

Acceptability of the analytical methods used in the test: Not applicable.

Endpoints: The 48-hour contact LD_{50} is > 87 µg a.s./bee (nominal), the highest dose tested. The NOEL is 87 µg a.s./bee.

Conclusion of the RMS: The honeybee acute oral toxicity test is considered valid.

b) Evaluation of additional data for the purpose of renewal of Annex I inclusion

No additional data was submitted or required for the purpose of renewal of approval of gibberellins GA4/GA7.

B.9.3.1.2. Acute contact toxicity to bees

a) Previous evaluation (2005-2011)

Two acute contact toxicity studies with gibberellins GA4/GA7 and gibberellic acid GA3 were submitted and evaluated as part of the EU review for the inclusion of GA4/GA7 in Annex I and are available in the EU DAR. These studies were considered acceptable in the EFSA conclusion for the acute contact risk assessment of bees. The critical study testing GA4/GA7 is considered appropriate for the current assessment to support renewal of GA4/GA7 and no new studies are submitted assessing the acute contact toxicity to bees. Full details of the study are provided in the EU DAR and related documents and references are listed in DRAR Vol.2.

PREVIOUS EVALUATION	This study was evaluated in the original DAR and has been considered by EFSA. No new evaluation has been performed. The conclusion has not been changed.
Data point addressed:	CA 8.3.1.1/02 (II A 8.3.1.1/02 in original DAR)
Author(s) (year):	Hoberg, J.R. (2004b)
Title:	Gibberellins A4 & A7 – Acute contact toxicity test with the honey bee (*Apis mellifera*)
Laboratory report / project number:	13828.6104
Testing facility:	Springborn Smithers Laboratories, Wareham, USA
Published:	No
Test guideline used:	OECD 214; EPPO 170 (1992)
Deviations:	None
GLP:	Yes
EU Agreed Endpoint:	48 h acute contact LD_{50} > 100 µg/bee

Executive summary

The acute contact toxicity of gibberellins (GA4/GA7) to the honey bee *Apis mellifera* was determined in a 48-hour test. The 48-hour contact LD_{50} is > 100 µg a.s./bee (nominal), the highest dose tested. The NOEC is considered to be 100 µg a.s./bee.

I MATERIALS AND METHODS

MATERIALS

Test material:	Gibberellins A4 + A7 (GA4/A7)
Lot/Batch No.:	107-554-CD
Purity:	90.3%
Stability of test compound:	Not reported, but may be considered to be adequately stable under the conditions of this study based on the behaviour observed in other tests.

Test organism:	*Apis mellifera*.
Age	Young adult workers taken from the brood frames of a mature hive.
Source:	Colonies maintained in hives at the test facility.
Treatments:	Seven groups: control and acetone solvent control and gibberellic acid treatments applied at nominal doses of 1.56, 3.13, 6.25, 12.5 and 25.0 µg/bee. A 1.0 µL droplet of the appropriate solution of gibberellins GA4/GA7 dissolved in acetone was administered to the thoracic surface of lightly anaesthetised bees, giving doses equivalent to 1.0, 10 and 100 µg a.s./bee. Solvent control bees were similarly dosed with acetone and control bees were subjected to the anaesthetisation process only. The study also included three further groups of bees dosed with the reference toxicant dimethoate at 0.050, 0.10 and 0.20 µg/bee.
Replication	Three chambers constructed of polyester mesh attached to a PVC frame (13 ×13 ×13 cm) per treatment group for the two controls, the highest test substance treatment and the dimethoate reference substance, two replicates for all other groups.

Number of bees per group:	30 (10 bees per replicate) for the controls, the highest test substance treatment and the reference substance; 20 (10 bees per replicate) for all other groups.
Duration:	48 hours.
Conditions:	The test units were housed inside an incubator with water and food (50% aqueous sucrose solution) available *ad libitum*.
Temperature:	25 to 26°C.
Relative humidity:	56% to 58%.
Photoperiod:	Continuous near-darkness.
Confirmatory analysis:	None.
Observations:	Bees were observed 4, 24 and 48 hours after dose application to count the numbers of mortalities and individuals exhibiting symptoms of toxicity or abnormal behaviour. A single observation at 24 h was made in the reference toxicant group.
Statistical analysis:	Not required.

II RESULTS AND DISCUSSION

A FINDINGS

Mortality in the control and solvent control groups was 0% and 3.3%, respectively, after 48 h. There were no mortalities in any of the GA4/GA7 treatment groups and all surviving bees appeared normal in behaviour throughout the study in the control and test substance groups. Surviving bees of the dimethoate reference treatments ≥0.10 µg/bee were lethargic at the 24-h observation.

B VALIDITY

Mortality in the control and solvent control groups was below 10%.

After 24 h, mortality in the three dimethoate dose groups ranged from 10% to 97%, indicating that the LD_{50} lay within the 0.10 to 0.30 µg/bee range stipulated by the test guideline.

III CONCLUSIONS

The acute contact toxicity of gibberellins (GA4/GA7) to the honey bee *Apis mellifera* was determined in a 48-hour test. The 48-hour contact LD_{50} is > 100 µg a.s./bee (nominal), the highest dose tested. The NOEC is considered to be 100 µg a.s./bee.

RMS comments and conclusion:

The study has been evaluated according to the OECD 214 Honeybee acute contact toxicity test. The OECD 214 toxicity test is designed to assess the acute contact toxicity of pesticides and other chemicals to adult worker honeybees.

Validity: According to the OECD 214 test guideline the honeybee acute contact toxicity test is considered acceptable if the following validity criteria are met:

- the average mortality for the total number of controls must not exceed 10 per cent at the end of the test **(in test: control mortality 0%, solvent control mortality 3.3%, condition fulfilled)**
- the LD_{50} of the toxic standard meets the specified range 0.10 – 0.30 µg/bee **(in test: mortality at 0.1 µg/bee dimethoate was 27%, mortality at 0.2 µg/bee dimethoate was 97%, condition fulfilled)**

The above summary provided by the applicant states that there were seven treatment groups: control and

acetone solvent control and gibberellic acid treatments applied at nominal doses of 1.56, 3.13, 6.25, 12.5 and 25.0 µg/bee. This is incorrect. There were in fact 5 treatment groups: control, solvent control (acetone) and 3 concentrations of gibberellins GA4/GA7 (1 µg/bee, 10 µg/bee and 100 µg/bee). Additionally, there were 3 treatment groups with toxic reference dimethoate (0.05 µg/bee, 0.1 µg/bee and 0.2 µg/bee).

This study was designed as preliminary range finding test with 3 concentrations of gibberellins GA4/GA7 that is 1 µg/bee, 10 µg/bee and 100 µg/bee. As there were no mortalities at 100 µg/bee the preliminary test qualified as limit test and no further testing was done.

Stability of the test compound was not tested in this study. The RMS agrees with the applicant that the test compound can be considered adequately stable based on the results of other studies submitted for re-registration.

Acceptability of the analytical methods used in the test: Not applicable.

Endpoints: The 48-hour contact LD_{50} is > 100 µg a.s./bee (nominal), the highest dose tested. The NOEC is 100 µg a.s./bee.

Conclusion of the RMS: The honeybee acute contact toxicity test is valid.

b) Evaluation of additional data for the purpose of renewal of Annex I inclusion

No additional data was submitted or required for the purpose of renewal of approval of gibberellins GA4/GA7.

B.9.3.1.3. Chronic toxicity to bees

a) Previous evaluation (2005-2011)

Chronic oral toxicity study is a new data requirement. The study was not submitted for the first inclusion of gibberellins GA4/GA7.

b) Evaluation of additional data for the purpose of renewal of Annex I inclusion

No data on the chronic toxicity of gibberellins GA4/GA7 to bees were submitted during the Annex I inclusion of GA4/GA7. A new study is submitted for the purposes of renewal, which is summarised below (CA 8.3.1.2/01) and references are listed in DRAR Vol.2.

Data point addressed:	CA 8.3.1.2/02
Author(s) (year):	Gray, J. (2017b)
Title:	Gibberellins A_4A_7: Honey bees (*Apis mellifera* L.) chronic oral toxicity test 10 day feeding in the laboratory
Laboratory report / project number:	FR30QH
Testing facility:	Envigo CRS Ltd., Huntingdon, UK
Published:	No
Test guideline used:	OECD proposal for a new guideline for the testing of chemicals; Honey bee chronic oral toxicity test 10 day feeding test in the laboratory, February 2016.
Deviations:	The humidity in the definitive test rose above 70% to a maximum of 70.80%. The mean humidity range was 62.90 – 63.79%. The temperature in the definitive test dropped below the minimum of 31°C to 29.3°C and rose above 35°C to a maximum of 36.5 °C. The mean temperature range was 31.1 – 32.9°C. These deviations were not considered by the Study Director to have had any adverse effect on the outcome of the study as mortality in the control groups was acceptable (≤15%). In addition, the mean temperature and humidity ranges were within acceptable limits.
GLP:	Yes
Endpoint:	10 d LD_{50} >150 mg a.s./kg 10 d NOEC = 150 mg a.s./kg 10 d LDD_{50} >5.644 µg a.s./bee/day 10 d NOEDD = 5.644 µg a.s./bee/day

Executive summary

A study was performed to determine the LC_{50} and LDD_{50} (median lethal dietary dose) values of Gibberellins GA4/GA7 to the honey bee (*Apis mellifera*) after 10 days continuous and *ad libitum* feeding. Five test concentrations were tested: 9.38, 18.75, 37.5, 75 and 150 mg a.s./kg. The survival and behaviour of bees were recorded after 4 hours and then daily up to 10 days, after the initial dose administration. The study was considered valid as control mortality was acceptable and administration of the toxic reference technical dimethoate resulted in substantial and unequivocal toxic effects. The 10-day LD_{50} value for GA4/GA7 to honey bees was estimated to be >150 mg a.s./kg. The NOEC was 150 mg a.s./kg. The 10-day LDD_{50} value for GA4/GA7 to honey bees was estimated to be >5.644 µg a.s./bee/day. The NOEDD was 5.644 µg a.s./bee/day.

I MATERIALS AND METHODS

A MATERIALS

Test item:	Gibberellins A4A7 Technical
Lot No.:	1000048922
Purity:	91.6% a.i.
Expiry date:	October 2016
Description:	White crystalline powder
Stability of test compound:	Adequately stable under the conditions of the study. Shown to be stable in bee diet formulations following storage at ambient temperature and under refrigeration for up to 4 days.

Toxic reference:	Dimethoate technical
Batch No.:	SZBC243XV
Purity:	99.5%
Expiry date:	August 2017
Description:	White crystalline solid
Test organism:	Sterile female worker honey bee (*Apis mellifera*)
Source:	Bees supplied by a local apiarist. Frames containing sealed brood were delivered to the testing facility prior to emergence of the adult female worker bees. After emergence, bees were inspected and considered to be in good health and free from disease.
Temperature:	Mean 31.1 – 32.9 °C
Photoperiod:	Maintained in darkness, except for essential procedures, which were conducted in subdued light.
Humidity:	Mean 62.90 – 63.79%

B STUDY DESIGN

After emergence ten bees were placed at random into each ventilated, glass-fronted stainless-steel cage (*ca*. 80 x 55 x 40 mm), without anaesthetisation. Newly emerged bees were removed from the comb up to 48 hours prior to dosing and were fed 50% w/v sugar solution.

The dose preparations were offered *ad libitum* to the honey bees via feeders (3 mL plastic syringes, tip removed) containing approximately 2 mL of the appropriate dispersion. The bees in each replicate shared the feeding solution (trophallaxis) and were therefore all expected to be exposed.

The study was conducted as a dose response test at 9.38, 18.75, 37.5, 75 and 150 mg a.s./kg. A control group of 50% w/v sucrose solution and a toxic reference group of 0.75 mg dimethoate/kg were also tested.

The feeding dispersions were replaced daily by replacing the dose with a new feeder containing freshly prepared test solution. Each feeding interval was 24 hours (< 30 minutes maximum). The amount of feeding solution consumed was determined daily by weighing the feeders before and after the feeding period.

To adjust for possible evaporation of the test preparations from the feeders, three additional test cages were set up in the definitive test containing no bees, only pre-weighed feeders containing diet of the sucrose control. These were placed in the test environment alongside the test units and weighed and replaced daily at the same time as the test feeders. The evaporation figure was then calculated and subtracted from the calculated food consumption to give corrected food consumption accounting for the loss by evaporation.

Chemical analysis of the test concentrations was conducted on Day 1.

Mortality was recorded daily at the same time of day (every 24 hours ± 2 hours), starting 24 ± 2 hours after the start of the test period. Behavioural abnormalities were also recorded at the same time. Behavioural abnormalities were quantitatively observed according to predetermined categories.

II RESULTS AND DISCUSSION

A FINDINGS

The measured concentrations in the dosing preparations for Day 1 were 8.44, 16.2, 34.5, 66.0 and 141 mg a.s./kg (90.0, 86.4, 92.0, 87.6 and 93.3 % of nominal).

Final mortality and food consumption is presented in the tables below.

Table 9.3.1.3/01- 1 : Summary of mortality for honey bees exposed to Gibberellins (GA4/GA7) (10 days)

Nominal concentration of test item (mg a.s./kg)	Cumulative mortality)*			Cumulative mortality (Mean %)
	Rep 1	Rep 2	Rep 3	
Control	0	0	0	0
9.38	0	0	1	3.3
18.75	0	0	0	0
37.5	0	0	0	0
75	0	0	1	3.3
150	0	0	0	0
Toxic reference	10	10	10	100

* Initial population = 10 per replicate

Mortality was not corrected as there was no mortality in the control group.
No significant effects of GA4/GA7 on honey bee mortality were observed at the concentrations tested.

Table 9.3.1.3/01- 2 : Summary of food consumption for honey bees exposed to Gibberellins (GA4/GA7) (10 days)

Nominal concentration of test item (mg a.s./kg)	Overall mean total consumption per bee (g)	Corrected mean total consumption per bee[a] (g)	Mean dose consumed per bee (µg a.s.)	Corrected mean dose consumed per bee[a] (µg a.s.)	% effect on consumption per bee
Control	0.3993	0.3380	-	-	-
9.38	0.4084	0.3471	3.8309	3.2558	+2.692
18.75	0.3359	0.2746	6.2977	5.1488	-18.76
37.5	0.3486	0.2873	13.0706	10.7738	-15.00
75	0.2998	0.2385	22.473	17.8875	-29.44
150	0.3761	0.3148	56.4395	47.2200	-6.86
Toxic reference	0.1198	0.1052	0.0899	0.0789	+53.58[b]

[a] Corrected for the evaporation blank – 0.0613 g

[b] during the <2-day survival period for the toxic reference

The 10-day LD_{50} (median lethal concentration) value was estimated to be >150 mg a.s./kg. The NOEC (no observed effect concentration) was 150 mg a.s./kg.
The 10-day LDD_{50} value (median lethal dietary dose) was estimated to be >5.644 µg a.s./bee/day. The NOEDD (no observed effect dietary dose) was 5.644 µg a.s./bee/day.

B VALIDITY

The test was considered valid as the following validity criteria were met:
- Average mortality in the control group was ≤15% at the end of the test (10 days following the start of the exposure period) (0%)
- Average mortality in the toxic reference group was ≥50% at the end of the test (10 days following the start of the exposure period) (100%)

III CONCLUSIONS

A study was performed to determine the LC_{50} and LDD_{50} (median lethal dietary dose) values of Gibberellins GA4/GA7 to the honey bee (*Apis mellifera*) after 10 days continuous and *ad libitum* feeding. Five test concentrations were tested: 9.38, 18.75, 37.5, 75 and 150 mg a.s./kg. The survival and behaviour of bees were recorded after 4 hours and then daily up to 10 days, after the initial dose administration. The study was considered valid as control mortality was acceptable and administration of the toxic reference technical

dimethoate resulted in substantial and unequivocal toxic effects. The 10-day LD_{50} value for GA4/GA7 to honey bees was estimated to be >150 mg a.s./kg. The NOEC was 150 mg a.s./kg. The 10-day LDD_{50} value for GA4/GA7 to honey bees was estimated to be >5.644 µg a.s./bee/day. The NOEDD was 5.644 µg a.s./bee/day.

RMS comments and conclusion:

The validity of this study was assessed in accordance with OECD 245 test guideline. The OECD 245 Honeybee (*Apis mellifera* L.) 10-day feeding chronic oral toxicity test is designed for determination of the LC_{50} (median Lethal Concentration), the LDD_{50} (median Lethal Dietary Dose) values after 10 days of exposure, NOEC (No Observed Effect Concentration) and NOEDD (No Observed Effect Dietary Dose).

Validity: According to the OECD 245 test guideline the honeybee chronic oral toxicity test is considered acceptable if the following validity criteria are met:

- The average mortality across replicates for the untreated control and solvent control groups is ≤ 15 % at the end of the test (10 days following start of exposure) **(in test: sucrose control mortality = 0%, condition fulfilled)**

- The average mortality in the reference substance treated group is ≥ 50 % at the end of the test (10 days following start of exposure) **(in test: dimethoate mortality = 100%, condition fulfilled)**

The humidity rose above 70% to a maximum of 70.80% (mean humidity range: 62.90 – 63.79%). The temperature dropped below the minimum of 31°C to 29.3°C and rose above 35°C to a maximum of 36.5 °C (mean temperature range: 31.1 – 32.9°C). The mortality in the control groups was acceptable (0%) and the mean temperature and humidity ranges were within acceptable limits. The RMS considers these deviations to be acceptable and it is unlikely that they had major effect on validity of the result.

Acceptability of the analytical methods used in the test: The analytical method for the determination of GA4/7 in aqueous sugar solution is considered valid and acceptable according to SANCO/3029/99 rev.4. No further data required. The assessment is provided in Section B.5.1.2.6.

Endpoints: The 10-day LD_{50} value for GA4/GA7 to honey bees is >150 mg a.s./kg. The NOEC is 150 mg a.s./kg. The 10-day LDD_{50} value for GA4/GA7 to honey bees is >5.644 µg a.s./bee/day. The NOEDD is 5.644 µg a.s./bee/day.

Conclusion of the RMS: The honeybee chronic oral toxicity test is considered to be valid.

B.9.3.1.4. Effect on honeybee development and other honeybee life stages

a) Previous evaluation (2005-2011)

Honeybee larval toxicity test is a new data requirement. The study was not submitted for the first inclusion of gibberellins GA4/GA7.

b) Evaluation of additional data for the purpose of renewal of Annex I inclusion

No data on the larval toxicity of gibberellins GA4/GA7 to bees were submitted during the Annex I inclusion of GA4/GA7. A new study is submitted for the purposes of renewal, which is summarised below (CA 8.3.1.3/01) and references are listed in DRAR Vol.2.

Data point addressed:	CA 8.3.1.3/01
Author(s) (year):	Taylor, K. (2017)
Title:	Gibberellins A_4A_7: Honey bee (*Apis mellifera*) larval toxicity test, single exposure
Laboratory report / project number:	CR15QN
Testing facility:	Envigo CRS Ltd., Huntingdon, UK
Published:	No
Test guideline used:	OECD 237 (2013)
Deviations:	None
GLP:	Yes
Endpoint:	72 h LD_{50} >100 µg a.s./larva

Executive summary

A study was performed to determine the acute toxicity of Gibberellins GA4/GA7 administered to honey bee (*Apis mellifera*) larvae with a single exposure. The study was conducted in accordance with OECD Guideline 237 (2013). Following an initial range finding phase the definitive study was conducted as a dose response test at 6.25, 12.5, 25, 50 and 100 µg a.s./larva. The survival of the larvae was recorded at 24, 48 and 72 hours after dose administration and food consumption was also monitored. The 72 h LD_{50} value for Gibberellins GA4/GA7 to honey bee larvae was estimated to be >100 µg a.s./larva. The study was considered valid as control mortality was acceptable (≤15%) and application of the toxic reference technical dimethoate at 8.8 µg a.s./larva resulted in >50% corrected mortality.

I MATERIALS AND METHODS

A MATERIALS

Test item:	Gibberellins A_4A_7 (GA4/GA7) Technical
Lot No.:	1000048922
Purity:	91.6% a.i.
Expiry date:	October 2016
Description:	White crystalline powder
Stability of test compound:	Not reported, but may be considered to be adequately stable under the conditions of this study based on the behaviour observed in other tests.

Toxic reference:	Dimethoate
Purity:	99.5 % a.i.
Lot No.:	SZBC243XV
Expiry date:	August 2017
Test organism:	Larval honey bee (*Apis mellifera*)
Source:	Bee larvae were supplied by an apiarist.
Age at test initiation:	4 days old
Temperature:	34.4 - 35.5 °C
Light regime:	Kept in the dark, except during feeding and assessments, which were conducted in subdued light

B STUDY DESIGN

On Day 1 individual larvae, one day old, were transferred from three hives into sterilised polystyrene grafting cells in 48-well plates by the apiarist. The cells, with an internal diameter of 9 mm and a depth of 8 mm, each contained 20 µL Diet A[1]. The cells were supported on a piece of dental roll placed at the bottom of each well and

wetted with 500 µL of sterilising solution in 15% w/v glycerol to maintain the rims of the grafting cells level with the upper surface of the plates. The larvae were transported to the laboratory in a heated box, at 28.6 to 29.6°C, containing moist tissue to provide a humid atmosphere. On arrival at the laboratory the plates containing the larvae were placed in a hermetic desiccator cabinet containing a dish filled with potassium sulphate saturated solution to maintain a water saturated atmosphere. The adapted desiccator was placed in a temperature-controlled incubator in darkness at 34 to 35°C.

On Day 3 the larvae were inspected and any dead were removed before surviving larvae were fed 20 µL Diet B[1] per cell. On each feeding occasion both pre and post dose administration both the feed and the 48-well plates were placed on a warming plate and the larvae were fed under subdued light. The feed was administered using a sterile pipette and the food was added along the wall of each grafting cell, taking care not to touch or drown the larvae.

On Day 3 twelve healthy larvae were selected for each replicate without conscious bias from the plates of larvae collected from one or more of three different hives as follows: Replicate 1 consisted of 12 larvae from hive A, Replicate 2 consisted of 12 larvae from hive B and Replicate 3 consisted of 12 larvae from hive C for all Gibberellins GA4/GA7 treatment rates and the water control in the range finder and additional range finder. Replicate 1 consisted of 12 larvae from hive A, Replicate 2 consisted of 12 larvae from hive B and Replicate 3 consisted of 12 larvae from hive C for all Gibberellins GA4/GA7 treatment rates, the water control and the toxic reference in the definitive study. After selection the grafting cells containing the larvae were transferred to clean multi-well plates. On Day 4 the larvae were checked to ensure 12 healthy larvae were present in each replicate and then treated with 30 µL of Diet C[1] containing the test or reference substance at the appropriate concentration.

Following two initial range finding tests the following rates were tested in the definitive test:

i. Water control
ii. Gibberellins GA4/GA7 administration of 6.25 µg a.s./larva
iii. Gibberellins GA4/GA7 administration of 12.5 µg a.s./larva
iv. Gibberellins GA4/GA7 administration of 25 µg a.s./larva
v. Gibberellins GA4/GA7 administration of 50 µg a.s./larva
vi. Gibberellins GA4/GA7 administration of 100 µg a.s./larva
vii. Dimethoate administration of 8.8 µg a.s./larva

Surviving larvae were fed 40 µL of Diet C[1] on Day 5 and 50 µL of Diet C on Day 6.

The criterion of effect employed in this study was mortality, determined by the absence of response to physical stimulation. The condition of each larva was recorded at the time of feeding on Days 5 and 6 and at termination of the test on Day 7. In addition to observations on mortality at 24, 48 and 72 hours after dose administration, the presence of uneaten food was also recorded. Cells containing dead larvae were removed from the test plates following the assessments on Days 5 and 6.

[1] Larval diet composition:
Diet A: 50% weight of fresh royal jelly plus 50% weight of an aqueous solution containing 2% weight of yeast extract, 12% weight of glucose and 12% weight of fructose.
Diet B: 50% weight of fresh royal jelly plus 50% weight of an aqueous solution containing 3% weight of yeast extract, 15% weight of glucose and 15% weight of fructose.
Diet C: 50% weight of fresh royal jelly plus 50% weight of an aqueous solution containing 4% weight of yeast extract, 18% weight of glucose and 18% weight of fructose.

Royal jelly for human consumption was used throughout. Batches RJ1502 (use by December 2016) and RJ1601 (use by May 2018) were used in the range finder and additional range finder respectively. Batch RJ1602 (use by end August 2018) was used in the definitive study.

II RESULTS AND DISCUSSION

A FINDINGS

The mean measured concentrations in Diet C were 0.199, 0.428, 0.982, 1.31 and 3.24 mg a.s./mL for treatment rates 6.25, 12.5, 25, 50 and 100 µg a.s./larva respectively, which are 95.2, 102.6, 117.7, 78.6 and 97.2% of nominal respectively. The mean concentrations were within applied limits ± 20% except for 50 µg a.s./larva which was within ± 25%. The biological results are based on nominal concentrations.

Cumulative mortality data for the definitive test are given in Table 9.3.1.4/01- 1 below.

Table 9.3.1.4/01- 1 : Summary of cumulative mortality data for honey bee larvae exposed to Gibberellins (GA4/GA7)

Nominal concentration of GA4/GA7 (µg a.s./larva)	Mortality (%)			Corrected mortality (%)		
	24 hours	48 hours	72 hours	24 hours	48 hours	72 hours
Water control	0	0	6	-	-	-
6.25	0	0	3	0	0	0
12.5	0	6	6	0	6	0
25	8	11	11	8	11	6
50	3	14	17	3	14*	12
100	6	17	17	6	17*	12
Toxic reference	50	69	92	50***	69***	91***

* Significant difference relative to the control ($P < 0.05$ using 1 tailed Fisher's exact tests)

*** Significant difference relative to the control ($P < 0.001$ using 1 tailed Fisher's exact tests)

The 72 h LD_{50} for Gibberellins GA4/GA7 to honey bee larvae was estimated to be >100 µg a.s./larva with the 95% confidence levels not estimable.

Reduced diet consumption was recorded after 24 hours for 0, 0, 3, 1 and 16 of the 36 larvae dosed at 6.25, 12.5, 25, 50 and 100 µg a.s./larva respectively and for an additional 1, 4 and 1 larvae at 25, 50 and 100 µg a.s./larva respectively at 48 hours. Mortality of 11.1, 13.9 and 16.7% was also recorded at 25, 50 and 100 µg a.s./larva in replicates where there had been reduced diet consumption. In the dimethoate treatment of 8.8 µg a.s./larva reduced diet consumption was recorded in 32 replicates after 24 hours and in the remaining 4 replicates at 48 hours. Mortality reached 91.7% after 72 hours.

B VALIDITY

The study was considered valid as control mortality was acceptable (≤15%) and application of the toxic reference technical dimethoate at 8.8 µg a.s./larva resulted in ≥50% corrected mortality.

III CONCLUSIONS

A study was performed to determine the acute toxicity of Gibberellins (GA4/GA7) administered to honey bee (*Apis mellifera*) larvae with a single exposure. The study was conducted in accordance with OECD Guideline 237 (2013). Following an initial range finding phase the definitive study was conducted as a dose response test at 6.25, 12.5, 25, 50 and 100 µg a.s./larva. The survival of the larvae was recorded at 24, 48 and 72 hours after dose administration and food consumption was also monitored. The 72 h LD_{50} value for Gibberellins GA4/GA7 to honey bee larvae was estimated to be >100 µg a.s./larva. The study was considered valid as control mortality was acceptable (≤15%) and application of the toxic reference technical dimethoate at 8.8 µg a.s./larva resulted in >50% corrected mortality.

RMS comments and conclusion:

The OECD 237 Honey bee (*Apis mellifera*) single exposure larval toxicity test aims at the determination of the

lethal dose seventy-two hours (72-h LD_{50}) following single exposure of larvae to a chemical (particularly pesticide active ingredient or formulation).

Validity: According to the OECD 237 test guideline the honeybee larval toxicity test is considered acceptable if the following validity criteria are met:

- In the control plate(s), cumulative larval mortality from D4 to D7 should be ≦ 15% across replicates **(in test: water control mortality = 6%, condition fulfilled)**
- In the reference chemical treatment, larval mortality (after adjustment) should be ≧ 50% at D7 **(in test: dimethoate mortality = 91%, condition fulfilled)**

The temperature in this study ranged from 34.5°C to 35.5°C which is outside of the range 34°C to 35°C as recommended by the test guideline. The RMS considers this to be a minor deviation that does not affect the validity of the result.

The test substance (gibberellins GA4/GA7) was dissolved in acetone; therefore, a solvent control should have been included in the study. The concentration of acetone in the final test solution was 5%, which is in line with the test guideline. The solvent control was included in preliminary range finding test, but not in the final definitive test. There were no mortalities in the solvent control in the preliminary test. The concentration of acetone in the final test solutions used in definitive test was equal to the concentration of acetone in the preliminary test. The RMS considers that it is unlikely that the mortality in solvent control of definitive test, had it been used, would exceed 15%. The study results are considered less reliable but still acceptable.

Acceptability of the analytical methods used in the test: The method was successfully validated for the determination of GA4/7 in Diet C formulation in accordance with SANCO/3029/99 rev. 4. The assessment is provided in Section B.5.1.2.6.

Endpoints: The 72 h LD_{50} is > 100 µg a.s./larva (nominal).

Conclusion of the RMS: The honeybee larval toxicity test is considered valid.

B.9.3.1.5. Sub lethal effects

No further data available.

B.9.3.2. Effects on non-target arthropods other than bees

B.9.3.2.1. Effects on Aphidius rhopalosiphi

a) Previous evaluation (2005-2011)

A toxicity test on *Aphidius rhopalosiphi* with gibberellins GA4/GA7 was submitted and evaluated as part of the EU review for the inclusion of GA4/GA7 in Annex I and is available in the EU DAR. The study was considered acceptable in the EFSA conclusion for the risk assessment of non-target arthropods. This study is considered appropriate for the current assessment to support renewal of GA4/GA7 and no new studies are submitted assessing the toxicity of the technical grade active substance to *Aphidius rhopalosiphi*. Full details of the study are provided in the EU DAR and related documents and references are listed in DRAR Vol.2.

PREVIOUS EVALUATION	This study was evaluated in the original DAR and has been considered by EFSA. No new evaluation has been performed. The conclusion has not been changed.
Data point addressed:	CA 8.3.2/01 (II A 8.3.2/01 in original DAR)
Author(s) (year):	Nienstedt, K.M. (1999a)
Title:	Gibberellin A4 & A7 – Toxicity test with the parasitic wasp, *Aphidius rhopalosiphi* (Hymenoptera: Braconidae)
Laboratory report / project number:	1042.008.270
Testing facility:	Springborn Smithers Laboratories (Europe) AG, Horn, Switzerland
Published:	No
Test guideline used:	Mead-Briggs (1992); Mead-Briggs (1997): draft guideline of the *Aphidius* ring-testing group; Polgàr (IOBC/WPRS) (1988)
Deviations:	None
GLP:	Yes
EU Agreed Endpoint:	LR_{50} > 40 g a.s./ha

Executive summary

The effects of gibberellins (GA4/GA7) to the parasitoid wasp *Aphidius rhopalosiphi* was determined in a Tier 1 laboratory test, with an initial 48-hour phase of exposure to dried spray deposits applied to glass plates. The 48-hour LR_{50} is > 40 g a.s./ha (nominal), the single limit rate tested.

I MATERIALS AND METHODS

MATERIALS

Test material:	Gibberellin A4/A7
Lot/Batch No.:	33263CD00
Purity:	72.5% Gibberellin A4 + 27.5% Gibberellin A7 (90.8% total Gibberellin A4/A7)
Stability of test compound:	Not reported, but may be considered to be adequately stable under the conditions of this study based on the behaviour observed in other tests.

Reference toxicant:	'Perfektthion'
Active ingredient:	dimethoate
Lot/batch No.:	E701002 (BASF)
Purity:	40.0%.
Test organism:	*Aphidius rhopalosiphi*.
Age	Adult, < 48 h post-emergence.
Source:	Supplied by PK Nützlingszuchten, Welzheim, Germany, as aphid mummies and hatched at the test facility.
Treatments:	Gibberellins (GA4/GA7) was applied at a rate of 40 g a.s./ha to glass plates using an automated sprayer and an application volume equivalent to 200 L/ha. In addition, a control treatment was prepared with deionised water only and a reference treatment was prepared with 'Perfekthion' (dimethoate) at a rate equivalent to 0.5 mL/ha. The test units consisted of two glass plates fitted to the top and bottom sections of a steel casing perforated with holes. The holes provided ventilation (through mesh covers), an entrance aperture for introducing the wasps that was later filled with a wick supplying a honey:water feeding solution, and an air suction attachment to purge any volatile substances from each test unit. After the sprayed residues had dried

	the test units were assembled with the treated surfaces of the plates facing inwards and the wasps introduced. Surviving wasps from the test units of the control and 40 g GA4/GA7/ha treatments were subsequently monitored through to the fecundity stage. 14 females selected at random were transferred individually to fecundity chambers comprising potted barley seedlings enclosed in an acrylic cylinder capped with mesh.
Replication	Four exposure units per treatment group for the initial exposure phase. Fourteen fecundity chambers for the control and test treatments for the subsequent reproduction stage.
Number of wasps per group:	40 (10 wasps per replicate) during the initial exposure phase. Survivors were housed singly for the initial 24 h of the subsequent parasitisation phase.
Duration:	Exposure: 48 hours; parasitisation: 24 h; mummy development: 10 days.
Temperature:	19 to 20.5°C.
Relative humidity:	73% to 90% %).
Photoperiod:	16 hours, intensity 1185 and 2312 to 2790 lux during the exposure and fecundity phases, respectively.
Confirmatory analysis:	None.
Observations:	Mortality and behavioural abnormalities were assessed 1, 24 and 48 hours after introduction of the wasps to the exposure units. Numbers of parasitized aphids that developed into mummies were counted at the end of the reproduction phase, 10 days after the 24-h oviposition phase.
Statistical analysis:	Not required to determine the LR_{50}. Shapiro-Wilk's test for normality, followed by a t-test for significance, was applied to the fecundity data.

II RESULTS AND DISCUSSION

A FINDINGS

There were no mortalities among the control wasps or among the wasps exposed to gibberellins (GA4/GA7). Mean mortality in the dimethoate treatment was 80% and 100% after 24 and 48 hours, respectively.

The mean numbers of parasitized aphids in the untreated controls and the gibberellins (GA4/GA7) treatment group were 21.7 and 26.8 and were not significantly different ($p > 0.05$). Exposure to 40 g gibberellins (GA4/GA7)/ha caused no adverse impact on fecundity.

There were no behavioural abnormalities among the wasps exposed to gibberellins (GA4/GA7) throughout the test.

B VALIDITY

Mortality in the control group was below 12.5%. Cumulative mortality in the dimethoate dose group exceeded 50% within 24 h.

III CONCLUSIONS

The effects of gibberellins (GA4/GA7) to the parasitoid wasp *Aphidius rhopalosiphi* was determined in a Tier 1 laboratory test, with an initial 48-hour phase of exposure to dried spray deposits applied to glass plates. The 48-hour LR_{50} is > 40 g a.s./ha (nominal), the single limit rate tested.

RMS comments and conclusion:

The study was conducted and evaluated according to the currently valid test guideline by Mead-Briggs *et al* (2000) A laboratory test for evaluating the effects of plant protection products on the parasitic wasp, *Aphidius rhopalosiphi* (DeStephani-Perez) (Hymenoptera: Braconidae). The method described by Mead-Briggs *et al* (2000) is designed to evaluate the effects in terms of mortality over 48h exposure period and sub-lethal effects

in terms of reproduction capacity of surviving females (10-12 days).

Validity: According to the test guideline by Mead-Briggs *et al* (2000) the study is considered valid if the following criteria are met:

- the mortality in the control treatment is below 13% (< 5 out of 40 wasp)**(in test: control mortality 0%, condition fulfilled)**
- the level of mortality expected in the toxic reference is in line with the mortality specified in the study protocol **(in test: toxic reference is dimethoate, mortality 80% at 24h, condition fulfilled)**
- the wasps in the control produce a minimum of 5 mummies per female. Lower fecundity levels would not allow a meaningful comparison of treatment. In the control, there should be no more than two wasps producing zero values (**in test: mean number of aphids per female in control = 21.7 ± 16, condition fulfilled).**

The humidity during the study was between 73% and 90%, which is outside of the recommended humidity range of 60 to 80%. The RMS considers this to be a minor deviation that does not affect the validity of the result.

Stability of the test compound was not tested in this study. The RMS agrees with the applicant that the test compound can be considered adequately stable based on the results of other studies submitted for re-registration.

Acceptability of the analytical methods used in the test: Not applicable.

Endpoints: The 48 h LR_{50} is > 40 g a.s./ha.

Conclusion of the RMS: The test is considered valid.

b) Evaluation of additional data for the purpose of renewal of Annex I inclusion

No additional data was submitted or required for the purpose of renewal of approval of gibberellins GA4/GA7.

B.9.3.2.1. Effects on Typhlodromus pyri

a) Previous evaluation (2005-2011)

A toxicity test on *Typhlodromus pyri* with gibberellins GA4/GA7 was submitted and evaluated as part of the EU review for the inclusion of GA4/GA7 in Annex I and is available in the EU DAR. The study was considered acceptable in the EFSA conclusion for the risk assessment of non-target arthropods. This study is considered appropriate for the current assessment to support renewal of GA4/GA7 and no new studies are submitted assessing the toxicity of the technical grade active substance to *Typhlodromus pyri*. Full details of the study are provided in the EU DAR and related documents and references are listed in DRAR Vol.2.

PREVIOUS EVALUATION	This study was evaluated in the original DAR and has been considered by EFSA. No new evaluation has been performed. The conclusion has not been changed.
Data point addressed:	CA 8.3.2/02 (II A 8.3.2/02 in original DAR)
Author(s) (year):	Nienstedt, K.M. (1999b)
Title:	Gibberellin A4 & A7 – Laboratory contact toxicity test with the predacious mite, *Typhlodromus pyri* Scheuten (Acari: Phytoseiidae)
Laboratory report / project number:	1042.008.268
Testing facility:	Springborn Smithers Laboratories (Europe) AG, Horn, Switzerland
Published:	No
Test guideline used:	Overmeer and Van Zon (1982); Overmeer (IOBC/WPRS) (1988); Baier et al. (1999) Draft *T.pyri* ring-test protocol
Deviations:	None
GLP:	Yes
EU Agreed Endpoint:	LR_{50} > 40 g a.s./ha

Executive summary

The effects of gibberellins (GA4/GA7) to the predatory mite *Typhlodromus pyri* was determined in a Tier 1 laboratory test, with exposure to dried spray deposits applied to glass slides. The LR_{50} is > 40 g a.s./ha (nominal), the single limit rate tested.

I MATERIALS AND METHODS

MATERIALS

Test material:	Gibberellin A4/A7
Lot/Batch No.:	33263CD00
Purity:	72.5% Gibberellin A4 + 27.5% Gibberellin A7 (90.8% total Gibberellin A4/A7)
Stability of test compound:	Not reported, but may be considered to be adequately stable under the conditions of this study based on the behaviour observed in other tests.

Reference toxicant:	'Perfektthion'
Active ingredient:	dimethoate
Lot/batch No.:	E701002 (BASF)
Purity:	40.0%.
Test organism:	*Typhlodromus pyri*.
Age	Protonymphs.
Source:	Stock culture maintained at the test facility, established with animals originally obtained from BASF, Limburgerhof, Germany.
Treatments:	Gibberellins (GA4/GA7) dissolved in acetone was applied at a rate of 40 g a.s./ha to glass plates using an automated sprayer and an application volume equivalent to 200 L/ha. In addition, a control treatment was prepared with deionised water only, a solvent control was treated with acetone and a reference toxicant was prepared with 'Perfekthion' (dimethoate) at a rate equivalent to 18 mL/ha. Each test unit consisted of a plastic container that housed a glass plate (10.3 × 5.0 cm), with the spray applied to a central 22.7 cm^2 portion of its upper surface. Each plate was placed over tissue that wicked moisture from a bed of wet cotton wool. A ring of sticky glue laid on the issue and around the perimeter of each glass plate was intended to prevent

	the mites escaping; the narrow gap between the glass edge and the glue permitted access to water. Walnut and apple pollen, placed within the confines of the glue barrier, was added to each test unit as food.
Replication	Five exposure units per treatment group.
Number of mites per group:	100 (20 mites per replicate).
Duration:	14 days.
Temperature:	24 to 27°C.
Relative humidity:	67% to 89% %).
Photoperiod:	16 hours, intensity 1133 to 1500 lux.
Confirmatory analysis:	None.
Observations:	Mortality (the number of dead, missing and escaped mites) was assessed 1, 3 and 7 days after the introduction of the protonymphs to the test units. On days 7, 9, 11 and 14, the numbers of males and females were recorded together with numbers of eggs and juvenile mites.
Statistical analysis:	Not required to determine the LR_{50}. Mortality data were analysed for significance with the Yates corrected Chi-square test. Shapiro-Wilk's test for normality, followed by ANOVA for significance, was applied to the fecundity data.

II RESULTS AND DISCUSSION

A FINDINGS

After Day 7 there were no significant differences (P > 0.05) between mortalities among the solvent control mites or among the mites exposed to gibberellins (GA4/GA7). Mean corrected mortality was -1.1% in the GA4/GA7 treatment and 83.2% in the dimethoate reference treatment.

Table 9.3.2.1/01- 1 : Mortality of *T. pyri* following 7-day exposure to gibberellins (GA4/GA7) on glass slides under laboratory conditions

Nominal application rate of gibberellins (GA4/GA7) (g a.s./ha)	Mean mortality after 7 days (out of 100 mites/treatment)			Mean corrected mortality (%) on Day 7
	Day 1	Day 3	Day 7	
0 (control)	2.0	8.0	15.0	4.5
0 (solvent control)	2.0	3.0	11.0	-
40	1.0	6.0	10.0	-1.1
reference toxicant	43.0	82.0	85.0	83.2

[a] Corrected mortality (%) = (Mt-Msc)/((100-Msc) × 100, where Mt = % mortality in the test treatment and Msc = mortality in the solvent control group.

[b] Not significantly different from the solvent control (p > 0.05).

[c] Excluded from statistical analysis.

The mean total numbers of eggs laid per female mite in the solvent control and the GA4/GA7 groups were 8.86 and 7.60 and were not significantly different. Exposure to 40 g gibberellins (GA4/GA7) caused no adverse effect on fecundity.

Table 9.3.2.1/01- 2 : Fecundity of *T. pyri* following 7-day exposure to gibberellins (GA4/GA7) on glass slides under laboratory conditions

Nominal application rate of gibberellins (GA4/GA7) (g a.s./ha)	Mean number of eggs per female				
	Day 7	Day 9	Day 11	Day 14	Total
0 (control)	2.07	2.08	2.49	3.10	9.74[a]
0 (solvent control)	1.11	1.96	2.15	3.63	8.86
40	1.21	1.77	2.07	2.56	7.60[a]
reference toxicant	0.00	0.25	0.25	0.00	0.50[b]

[a] Not significantly different from the solvent control ($p > 0.05$).

[b] Based on only two replicates with female survivors, excluded from statistical analysis.

B VALIDITY

Mortality in the control group was below 20% after 7 days and the control group produced more than 4 eggs/female during the fecundity phase. Cumulative mortality in the dimethoate dose group exceeded 50% within 7 days.

III CONCLUSIONS

The effects of gibberellins (GA4/GA7) to the predatory mite *Typhlodromus pyri* was determined in a Tier 1 laboratory test, with exposure to dried spray deposits applied to glass slides. The LR_{50} is > 40 g a.s./ha (nominal), the single limit rate tested.

RMS comments and conclusion:

Test guideline The Laboratory residual contact test with the predatory mite *Typhlodromus pyri* Scheuten (Acari: Phytoseiidae) for regulatory testing of plant protection products by Blümel et al (2000) aims to evaluate lethal and sub-lethal effects of a test item (spray formulation) on the predatory mite *T.pyri*.

Validity: According to the test guideline the test is considered acceptable if the following validity criteria are met:

- The arithmetic mean mortality (dead and escaped individuals in the control should not exceed 20% on day 7 after treatment application **(in test: control mortality = 15%, solvent control mortality = 11%, condition fulfilled)**

- The cumulative mean number of eggs per female in the control (from day 7 to day 14) should be ≥ 4 eggs/female **(in test: control mean no. eggs/female = 9.7, solvent control mean no. eggs/female = 8.86, condition fulfilled)**

- The cumulative mean mortality (control corrected) of protonymphs on day 7 exposed to toxic reference item should range between 50% and 100% **(in test: dimethoate mortality = 83.2%, condition fulfilled)**

Stability of the test compound was not tested in this study. The RMS agrees with the applicant that the test compound can be considered adequately stable based on the results of other studies submitted for re-registration.

Acceptability of the analytical methods used in the test: Not applicable.

Endpoints: The 7-day LR_{50} is > 40 g a.s./ha, the maximum rate tested.

Conclusion of the RMS: The test is considered valid.

b) Evaluation of additional data for the purpose of renewal of Annex I inclusion

No additional data was submitted or required for the purpose of renewal of approval of gibberellins GA4/GA7.

B.9.4. EFFECTS ON NON-TARGET SOIL MESO- AND MACROFAUNA

B.9.4.1. Earthworm – sub-lethal effects

a) Previous evaluation (2005-2011)

Reproduction study with earthworm was not submitted for the first inclusion of gibberellins GA4/GA7. No data gap was identified in EFSA conclusions (EFSA Journal 2012;10(1):2502) as low risk was concluded based on low persistency in soil.

An acute toxicity test on *Eisenia fetida* with GA4/GA7 was submitted and evaluated as part of the EU review for the inclusion of GA4/GA7 in Annex I and is available in the EU DAR. The study was considered acceptable in the EFSA conclusion for the risk assessment of earthworms. The acute earthworm risk assessment scheme according to SANCO/10329/2002 rev 2 final is now obsolete, but the acute data is included here for information to support the low toxicity of GA4/GA7 to earthworms.

PREVIOUS EVALUATION	This study was evaluated in the original DAR and has been considered by EFSA. No new evaluation has been performed. The conclusion has not been changed.
Data point addressed:	CA 8.4.1/01 (II A 8.4.1/01 in original DAR)
Author(s) (year):	Porch, J.R. & Krueger, H.O. (2001)
Title:	ABG-3192 [Gibberellin A4 + A7]: An acute toxicity study with the earthworm in an artificial soil substrate
Laboratory report / project number:	529-103
Testing facility:	Wildlife International Ltd., Maryland, USA
Published:	No
Test guideline used:	OECD 207
Deviations:	The initial group mean body weight was 294 mg, slightly below the 300 mg minimum indicated by the test guideline, but this is not likely to have influenced the outcome of the study
GLP:	Yes
EU Agreed Endpoint:	14 d LC_{50} > 1250 mg a.s./kg dw soil LC_{50CORR} > 625 mg a.s./kg dw soil (in accordance with SANCO/10329/2002 rev 2 final, as the log P_{ow} > 2, the toxicity endpoint was divided by 2 to take account of the different amount of organic carbon between laboratory and natural soils)

Executive summary

The 14-day LC_{50} of gibberellins (GA4/GA7) to *Eisenia fetida*, based on nominal initial concentrations, was greater than 1250 mg/kg dry soil. Based on the absence of treatment-related body weight effects or abnormalities in behaviour or appearance, the short-term NOEC was 1250 mg/kg dw soil.

I MATERIALS AND METHODS

MATERIALS

Test material:	Gibberellin A4 + A7, GA 4/7
Lot/Batch No.:	57-601 CD00
Purity:	90.0% (w/w)
Stability of test compound:	Not reported.

Test organism:	*Eisenia fetida*.
Age	Adult, with clitellum, acclimated to artificial test soil for 24 h under laboratory conditions prior to test initiation. Replicate group bodyweight range at initiation: 280 to 320 g, mean 294 g.
Source:	Stock culture maintained at the test facility.
Treatments:	Six groups: control (OECD artificial soil containing 10% peat) and soil-incorporated gibberellins (GA4/GA7) treatments applied at nominal concentrations of 78, 156, 313, 625 and 1250 mg/kg dry weight. At test initiation a weighed group of 10 randomly allocated worms was placed on the top of the soil surface in each container.
Replication	Four 1 L glass beakers, each containing 750 g soil, per treatment.
Number of worms per group:	40 (10 per replicate).
Duration:	14 days.
Conditions:	All test vessels were covered with perforated plastic film. The worms were not fed during the test.
Temperature:	20 to 22°C.
Soil moisture content:	32.0% to 32.7% on day 0, 30.4% to 32.1% on Day 14.
Soil pH:	7.0 to 7.1 on Day 0; 6.9 to 7.0 on Day 14.
Photoperiod:	Continuous, mean intensity 543 lux.
Confirmatory analysis:	None.
Observations:	Burrowing behaviour was monitored at test initiation to check for substrate avoidance reactions. On Day 7 the containers were emptied to count the numbers of dead and surviving worms and to check for pathological abnormalities. The containers were then re-packed with their respective contents and surviving worms returned to the soil surface and the burrowing assessment repeated. Final counts were made on Day 14 and the worms retrieved from each container were rinsed, blotted dry and re-weighed.
Statistical analysis:	Not required to determine the 14-d LC_{50} or the mortality NOEC. Dunnett's test was used to identify differences in group body weights and bodyweight changes between the gibberellins GA4/GA7 treatments and the control.

II RESULTS AND DISCUSSION

A FINDINGS

A single mortality (2.5%) was recorded on Day 7 in the control group. Mortality in all the gibberellins (GA4/GA7) treatment groups was either less than or equal to that in the control group on Day 14. As mortality was below 50% in all test substance treatments, the LC_{50} could not be calculated, but it exceeded1250 mg/kg dry soil, the highest concentration tested.

Group mean body weights ranged between 0.29 and 0.30 g at the start of the test and were reduced to between 0.324 and 0.26 g on day 14, consistent with the fact that no food was provided during the test. There were no statistically significant ($p > 0.05$) differences between group mean weights recorded for the control and the gibberellins (GA4/GA7) treatments at the start and end of the test, or between bodyweight changes that occurred

between Days 0 and 14. Worms of all treatments burrowed readily into the soil at the start of the test and on day 7. There were no differences in appearance of the worms between the control and test substance groups.

III CONCLUSIONS

The 14-day LC_{50} of gibberellins (GA4/GA7) to *Eisenia fetida*, based on nominal initial concentrations, was greater than 1250 mg/kg dry soil. Based on the absence of treatment-related body weight effects or abnormalities in behaviour or appearance, the short-term NOEC was 1250 mg/kg dw soil.

RMS comments and conclusions:

The OECD test guideline 207 Earthworm acute toxicity test is designed to be used for assessing the effects of test chemicals in soil on the mortality of the earthworm species *Eisenia fetida* or *Eisenia andrei.*

Validity: According to the OECD 207 test guideline the following criteria should be satisfied in the controls for a test result to be considered valid:

- The mortality in the controls should not exceed 10 per cent at the end of test **(in test: control mortality = 2.5%, condition fulfilled)**

The initial group mean body weight was 294 mg, slightly below the 300 mg minimum required by the test guideline. The RMS considers this to be acceptable, as the results of this study are used only as supplementary information.

Stability of the test compound was not tested in this study.

Acceptability of the analytical methods used in the test: Not applicable.

Endpoints: The 14 h LC_{50} is > 1250 mg a.s./kg dw soil. $LC_{50\ corr}$ > 625 mg a.s./kg dw soil ($logP_{ow}$ of gibberellins GA4/GA7 is above 2, therefore according to SANCO/10329/2002 rev 2 final the toxicity endpoint has to be divided by 2).

Conclusion of the RMS: The test is considered reliable and is used as supplementary information.

b) Evaluation of additional data for the purpose of renewal of Annex I inclusion

No data on the chronic toxicity of gibberellins GA4/GA7 to earthworms were submitted during the Annex I inclusion of GA4/GA7. A new chronic earthworm study testing the effects of GA3 is available, which is considered appropriate for the risk assessment of GA4/GA7 based on the similarities between GA3 and GA4/GA7, as well as the high margin of safety obtained in the risk assessment (see DRAR Vol.3 Novagib B.9.7). The new study submitted for the purposes of renewal is summarised below (CA 8.4.1/02) and references are listed in DRAR Vol.2.

Data point addressed:	CA 8.4.1/02
Author(s) (year):	Sloman, T.L. and Porch, J.R. (2017)
Title:	Gibberellic acid: a reproduction study with the earthworm in an artificial soil substrate
Laboratory report / project number:	529P-109
Testing facility:	EAG Laboratories, Maryland, USA
Published:	No
Test guideline used:	OECD 222; ISO 11268-2
Deviations:	None
GLP:	Yes
Endpoint:	EC_{50} (reproduction) >1000 mg a.s./kg dw soil EC_{20} (reproduction) >1000 mg a.s./kg dw soil EC_{10} (reproduction) = 316.5 mg a.s./kg dw soil NOEC (reproduction) = 250 mg a.s./kg dw soil

Executive summary

The effects of Gibberellic acid (GA3) on the reproductive output of the earthworm, *Eisenia fetida*, were tested in a 56-day dose-response study in an artificial soil substrate. Adults were exposed for 28 days and then removed to evaluate mortality and growth. The cocoons and the soil were returned to test chambers for an additional 28 days to evaluate effects on reproductive output (number of juveniles at test termination). Nominal test substance concentrations tested were 62.5, 125, 250, 500, and 1000 mg a.s./kg dw soil, as well as a negative control. There was no mortality of adult earthworms exposed to concentrations up to 1000 mg a.s./kg dw soil for 28 days. Based on body weight and survival data of adult earthworms, the no observed effect concentration (NOEC) was determined to be 1000 mg a.s./kg dry soil, the highest concentration tested. There were no reductions of ≥20% for the numbers of juveniles produced in the treatment groups compared to the control, therefore the EC_{20} and EC_{50} for reproduction were both >1000 mg a.s./kg dw soil. The EC_{10} for reproduction was determined to be 316.5 mg a.s./kg dw soil and the NOEC was 250 mg a.s./kg dw soil, based on juvenile production.

I MATERIALS AND METHODS

A MATERIALS

Test item:	Gibberellic acid A_3
Lot No.:	237979S4
CAS No.:	77-06-5
Purity:	92.88% (w/w)
Expiry date:	July 2019
Description:	Solid
Stability of test compound:	Not reported.

Test organism:	Earthworms (*Eisenia fetida*)
Source:	EAG Laboratories- Easton cultures started with earthworms originally obtained from the University of Maryland Wye Research and Education Center, Queenstown, Maryland.
Age at test initiation:	Adult earthworms with clitellum from a synchronous culture (individuals not differing in age by more than four weeks). Between 2 and 6 months old.

Test conditions

Test soil:	Artificial soil (70% sand, 20% kaolin clay, 10% sphagnum peat, 1% calcium carbonate)
Organic carbon content:	1.0% (organic matter content of 1.8%)
Soil pH:	7.4 – 7.6 at test initiation; 7.1 – 7.3 at test termination
Soil moisture content:	33.1 – 33.6% at test initiation; 34.1 – 36.4% at test termination
Soil temperature:	20.9 – 21.3 °C at test initiation; 21.1 – 21.2 °C at test termination
Air temperature:	20 – 22 °C
Photoperiod:	16 h light; 8 h darkness
Light intensity:	Mean 566 ± 46.7 lux

B STUDY DESIGN

Adult earthworms (2 - 6 months old) were transferred to the study room and held in a glass aquarium of conditioning bedding (peat) with cow manure as the food source for acclimation to test conditions for 7 days prior to conditioning in the artificial soil substrate. One day prior to test initiation, the adult earthworms were removed from the glass aquarium and divided equally into ten 1 L glass beakers each containing ~780 g artificial soil substrate adjusted to a moisture content of approximately 34% by weight, for the soil acclimation period. Each beaker was covered with perforated plastic wrap secured with a rubber band. Earthworms were fed cow manure throughout the acclimation period and held under the same environmental conditions as the test.

Test soils were prepared by mixing the appropriate amount of test substance with dry artificial soil before adding the finely ground cow manure and the RO water to hydrate the soil. Test soil components were mixed for approximately 20 minutes in order to achieve a homogeneous mixture. Negative control soil was similarly prepared but without the addition of test substance. 750 g of prepared soil were added to each of the test chambers (four chambers (replicates) per treatment group; eight control replicates). Nominal test substance concentrations tested were 62.5, 125, 250, 500, and 1000 mg a.s./kg dw soil.

On the day of test initiation, the earthworms were indiscriminately distributed into holding vessels by pairs into groups of ten earthworms each, rinsed briefly with RO water, gently blotted dry, weighed in groups of 10, and then placed randomly on the soil surface of a 1 L test chamber. To minimise bias, which might arise from the selection process, the test chambers were placed randomly on the counter in the test room. The earthworms were fed cow manure during testing. On Day 1 of the test, approximately 5 g of finely ground cow manure for food, and water to moisten the food, were added to the test chambers. Food was provided approximately weekly during the next three weeks by adding food in a small depression in the soil surface and covering it with a thin layer of soil. The amount of food supplied was reduced if uneaten food remained from the previous feeding interval. On Day 28, after adult earthworms were removed, approximately 5 g of additional manure were gently mixed into the test soil before it was returned to the test chambers.

Test chambers were weighed periodically to monitor soil moisture loss. Lost soil moisture was replaced by adding RO water to the soil surface until weights approximated those at the start of the test (Day 1) or those weights collected on Day 28 of the test (after adult earthworms were removed).

Observations and Measurements

At test initiation, the earthworms were placed on the surface of the soil in each replicate test chamber and were observed for burrowing behaviour. On Day 28, the test soil in each replicate chamber was removed and spread out onto non-absorbent paper to determine the number of surviving adult earthworms. All surviving adult earthworms were removed and observed for behavioural or pathological abnormalities and response to mechanical stimulus. Group body weights were measured for earthworms in each test chamber and the mean individual body weights were calculated. By folding the paper into a cylinder, the test soil containing any

cocoons and juveniles was gently returned to the test chambers. Following observations and body weight determinations, surviving earthworms were euthanized by freezing.

Juveniles were removed from the test soil on Day 56 and counted on Day 57. All holding containers were covered with perforated lids and kept in the study room prior to counting. The juveniles were removed from each replicate holding container on Day 57, counted, and any observed mortality and behavioural or clinical signs were documented.

II RESULTS AND DISCUSSION

A FINDINGS

A summary of the effects on adult mortality and mean body weights during the 28-day adult exposure period, as well as the reproductive output after 56 days, is presented in the table below.

Table 9.4.1/02- 1 : Summary of effects on earthworms following exposure to Gibberellic acid (GA3) in an artificial soil substrate

Test concentration (mg a.s./kg dw soil)	% Adult Mortality (Day 28)	Mean adult body weights ± SD (g)			Mean number of juveniles per replicate ± SD (Day 56)
		Day 0	Day 28	Increase (initial to final weight)	
Control	0	0.42 ± 0.013	0.52 ± 0.024	0.10 ± 0.029	200 ± 21.5
62.5	0	0.42 ± 0.008	0.52 ± 0.017	0.10 ± 0.010	199 ± 30.4
125	0	0.42 ± 0.008	0.52 ± 0.013	0.10 ± 0.010	197 ± 22.0
250	0	0.43 ± 0.032	0.52 ± 0.027	0.09 ± 0.025	178 ± 23.8
500	0	0.43 ± 0.015	0.51 ± 0.024	0.08 ± 0.015	172 ± 5.9*
1000	0	0.46 ± 0.024	0.53 ± 0.033	0.07 ± 0.026	163 ± 11.5*

* Statistically significant difference ($p<0.05$) between the treatment group and the control group with Williams multiple comparison test of means.

There were no mortalities in any of the treatment groups during the 28-day adult exposure period. All surviving earthworms in the negative control and the treatment groups were normal in appearance and behaviour and earthworms showed no aversion to test soils. The 28 d LC_{50} was determined to be >1000 mg a.s./kg dw soil.

Mean final body weight and change in body weight were compared to the control group mean using Dunnett's two-tailed test of means ($p=0.05$). There were no statistically significant differences between mean final body weight, and change in body weight, for the treatment groups when compared to the control group mean. Therefore, the NOEC (body weight) was determined to be 1000 mg a.s./kg dw soil.

The juveniles collected from the negative control and treatment groups were normal in appearance and behaviour. There was a statistically significant difference between the mean number of juveniles for the 500 and 1000 mg a.s./kg dw soil treatment groups when compared to the control using Williams multiple comparison test ($p>0.05$). Therefore, the NOEC for reproduction was determined to be 250 mg a.s./kg dw soil and the EC_{10} (reproduction) was determined to be 316.5 mg a.s./kg dw soil. There were no reductions of ≥20% for the numbers of juveniles produced in the treatment groups compared to the control, therefore the EC_{20} and EC_{50} for reproduction were both >1000 mg a.s./kg dw soil.

EAG Laboratories- Easton conducted a reference toxicity test with carbendazim in 2006 to document that the earthworms being cultured were sensitive to a known toxicant. The LC_{50} value for the mortality of the adult earthworms exposed to carbendazim for 28 days was 7.149 mg a.s./kg dw soil, with a 95% confidence interval of 6.338 and 8.273 mg a.s./kg dw soil. There were statistically significant ($p<0.01$) treatment-related losses in body weight among surviving adult earthworms at test concentrations of 2, 4, and 8 mg a.s./kg dw soil on Day 28. The

EC_{50} value for reproduction was 0.8914 mg a.s./kg dw soil, with a 95% confidence interval of 0.8416 and 0.9718 mg a.s./kg dw soil. The NOEC (reproduction) was 0.5 mg a.s./kg dw soil.

B VALIDITY CRITERIA

The test was considered to be acceptable based on the validity criteria. Adult mortality of negative control earthworms was less than 10%, with no mortalities occurring in the negative control group. There were 160 or more juveniles produced in each of the eight replicates for the negative control group, thereby meeting the criterion of 30 or more juveniles per container. The coefficient of variation of reproduction in the negative control group was 10.7%, thus satisfying the validity criterion of not exceeding 30%.

III CONCLUSIONS

The effects of Gibberellic acid (GA3) on the reproductive output of the earthworm, *Eisenia fetida*, were tested in a 56-day dose-response study in an artificial soil substrate. There was no mortality of adult earthworms exposed to concentrations up to 1000 mg a.s./kg dw soil for 28 days. Based on body weight and survival data of adult earthworms, the no observed effect concentration (NOEC) was determined to be 1000 mg a.s./kg dry soil, the highest concentration tested. There were no reductions of ≥20% for the numbers of juveniles produced in the treatment groups compared to the control, therefore the EC_{20} and EC_{50} for reproduction were both >1000 mg a.s./kg dw soil. The EC_{10} for reproduction was determined to be 316.5 mg a.s./kg dw soil and the NOEC was 250 mg a.s./kg dw soil, based on juvenile production.

RMS comments and conclusion:

The OECD 222 Earthworm Reproduction Test (*Eisenia fetida/ Eisenia andrei)* is designed to be used for assessing the effects of test chemicals in soil on the reproductive output (and other sub-lethal end points) of the earthworm species *Eisenia fetida* or *Eisenia andrei.*

Validity: According to the OECD 222 test guideline the following criteria should be satisfied in the controls for a test result to be considered valid:

- each replicate (containing 10 adults) to have produced ≥ 30 juveniles by the end of the test **(in test: mean number of juveniles in the control group = 200 ± 21.5, condition fulfilled)**
- the coefficient of variation of reproduction to be ≤ 30 % **(in test: reproductive coefficient of variation = 10.7%, condition fulfilled)**
- adult mortality over the initial 4 weeks of the test to be ≤10 % **(in test: control mortality = 0%, condition fulfilled)**

The test guideline recommends loading of 10 earthworms in 500 – 600 g of artificial soil. If larger quantities of soil are used the number of earthworms should be increased to maintain the loading of 50 – 60 g of soil per earthworm. In this study the loading was 10 earthworms in 750 g of soil. The RMS considers this to be a minor deviation.

The test guideline dictates the use of toxic reference substance, which can be either carbendazim or benomyl. Significant effects on reproduction should be observed between 1 and 5 mg a.i./kg dry mass. In this study the EC_{50} value for carbendazim for reproduction was 0.8914 mg a.i./kg dry soil, with a 95% confidence interval of 0.8416 and 0.9718 mg a.i./kg dry soil. The RMS considers this to be acceptable.

The OECD 222 test guideline states that eight treatment concentrations in a geometric series should be used to determine both NOEC and EC_x. The concentrations should be spaced by factor not exceeding 1.8. For

determination of NOEC a geometric series of five concentrations spaced by factor not exceeding 2 suffices. The design of this study is appropriate for determination of NOEC, but not EC_x. The NOEC value will be used for risk assessment, therefore, the RMS considers this study to be acceptable.

Stability of the test compound was not tested in this study.

Acceptability of the analytical methods used in the test: Not applicable.

Endpoints: The NOEC is 1000 mg a.s./kg dry soil, the highest concentration tested. The NOEC is 250 mg a.s./kg dw soil, based on juvenile production.

Conclusion of the RMS: The earthworm reproduction test is considered valid.

B.9.4.2. Effects on non-target soil meso- and macrofauna (other than earthworms)

Data are available on both *Aphidius rhopalosiphi* and *Typhlodromus pyri* (see CA 8.3.2) and no concerns are raised with either of these species (MCP 10.3.2). In accordance with the data requirements set out by EU Reg. 283/2013, no further data on effects on non-target soil meso- and macrofauna (other than earthworms) are considered necessary.

B.9.5. EFFECTS ON SOIL NITROGEN TRANSFORMATION

a) Previous evaluation (2005-2011)

A study testing the effects of gibberellins GA4/GA7 on nitrification of soil microflora was submitted and evaluated as part of the EU review for the inclusion of GA4/GA7 in Annex I and is available in the EU DAR. The study was considered acceptable in the EFSA conclusion for the risk assessment of soil microorganisms. This study is considered appropriate for the current assessment to support renewal of GA4/GA7 and no new studies are submitted assessing the toxicity of the technical grade active substance to soil microorganisms. Full details of the study are provided in the EU DAR and related documents and references are listed in DRAR Vol.2.

PREVIOUS EVALUATION	This study was evaluated in the original DAR and has been considered by EFSA. No new evaluation has been performed. The conclusion has not been changed.
Data point addressed:	CA 8.5/01 (II A 8.5/01 in original DAR)
Author(s) (year):	van der Kolk, J. (2000)
Title:	Gibberellin A4 & A7 – The effects on the respiration and nitrification of soil microflora
Laboratory report / project number:	1042.006.748
Testing facility:	Springborn Smithers Laboratories (Europe) AG, Horn, Switzerland
Published:	No
Test guideline used:	BBA, Part VI, 1-1 (1990)
Deviations:	None, according to the cited guidance. The guidance current at the time this study was performed has been superseded by OECD TG 216, which sets a ±25% divergence from the control under laboratory test conditions as the threshold of biological significance in terms of impact on nitrogen transformation in the field. The threshold applied in the current study was a divergence of ±15% from the control – a more stringent criterion than that set by the OECD TG. This deviation has no impact on the validity or reliability of the study.
GLP:	Yes
EU Agreed Endpoint:	Application of gibberellins (GA4/GA7) at a concentration of 0.013 mg/kg dry soil had no biologically significant effect on the transformation of organic nitrogen in either of two soils tested. Application at the higher concentration of 0.13 mg/kg dry soil suppressed the mineralisation and subsequent nitrification of organic nitrogen by the indigenous soil microflora in both soils. This effect was transient and the differences between these processes in the presence and absence of the test substance had diminished to less than 15% within 28 days. Gibberellins (GA4/GA7) caused no permanent effect on nitrogen transformation processes in soils at the concentrations applied.

Executive summary

Application of gibberellins (GA4/GA7) at a concentration of 0.013 mg/kg dry soil had no biologically significant effect on the transformation of organic nitrogen in either of two natural field soils. Application at the higher concentration of 0.13 mg/kg dry soil initially suppressed the mineralisation sand subsequent nitrification of organic nitrogen by the indigenous microflora in both soils. This effect was transient and the differences between these processes in the presence and absence of the test substance had diminished to less than 15%

within 28 days. Gibberellins (GA4/GA7) caused no permanent effect on nitrogen transformation in soils at the concentrations applied.

I MATERIALS AND METHODS

MATERIALS

Test material:	Gibberellin A4/A7
Lot/Batch No.:	33263CD00
Purity:	72.5% Gibberellin A4 and 18.3% Gibberellin A7 (90.8% total Gibberellin A4/A7)
Stability of test compound:	Not reported.

Test soils:

	Soil 1	**Soil 2**
Classification:	Loamy sand	Sandy loam
Source:	Sevelen, Switzerland	Untereggen, switzerland
Sampling depth:	8-20 cm (turf removed)	5-20 cm (turf removed)
Site history at time of soil collection:	Meadow for ≥2 years, no pesticides applied during previous 2 years, no mineral fertilisers during previous year and no organic fertiliser during the previous 6 months.	Meadow for ≥30 years, no pesticides applied during previous 2 years, no mineral or organic fertilisers during previous year.
Pre-treatment:	Sieved to 2 mm	Sieved to 2 mm
Acclimation:	Stored for 14 days at room temperature	Stored for 23 days at room temperature
pH	7.5	6.9
Organic carbon content:	0.92%	2.01%
Total nitrogen content:	120 mg/100 g	240 mg/100 g
Maximum water holding capacity (MWHC):	44.8 g/100 g dw soil	55.0 g/100 g dw soil
Microbial biomass:	Initial: 46.2 mg C/100 g dw soil; Final: 28.0 mg C/100 g dw soil (≡ 5.0% and 3.0% of C_{org}, respectively).	Initial: 69.1 mg C/100 g dw soil; Final: 72.5 mg C/100 g dw soil (≡ 3.4% and 3.6% of C_{org}, respectively).
Test organism:	Mixed population of indigenous soil microflora.	
Treatments:	Gibberellins (GA4/GA7), dissolved in acetone and plated onto a quartz sand carrier by evaporating the solvent, was incorporated at rates of 0 (solvent control), 0.013 and 0.13 mg/kg dry soil into a loamy sand (soil 1) and a sandy loam (soil 2). Batches of soil (6 kg dw) were prepared at each GA4/GA7 treatment rate by mixing with 60 g portions of sand treated with 5.0 mL volumes of the appropriate test substance stock solution. The control batches were similarly prepared by adding sand treated with acetone alone. Samples of the treated soil (100 g dry weight equivalents) were transferred from each batch into incubation flasks and each portion was mixed with 0.5 5 lucerne meal (N-content: 3.25%). A separate reference study was performed with 125 mg dinoseb acetate/kg dry loamy sand soil.	
Replication	24 replicates per treatment for both soils.	

Duration:	28 days.
Temperature:	18 to 21°C.
Soil moisture content:	41% MWHC (soil 1); 40% MWHC (soil 2).
Soil pH:	7.37 to 7.59 at initiation, 7.45 to 7.54 at termination (soil 1); 6.90 to 6.92 at initiation; 7.02 to 7.09 at termination (soil 2).
Photoperiod:	Not reported.
Confirmatory analysis:	None.
Observations:	On Days 0, 14 and 28, sub-samples (20 g dry weight equivalents) were removed and extracted with 0.1 M aqueous KCl solution. The extracts were centrifuged and filtered (both to exclude undissolved material and to eliminate chloride interference), then analysed by ion chromatography to determine concentrations of nitrite and nitrate.
Statistical analysis:	the Dixon test was used to identify outliers among replicates and to justify their exclusion from the calculation of mean values. Student's t-test was applied to test for statistically significant ($p = 0.05$) differences between the gibberellin (GA4/GA7) treatments and the appropriate control values.

II RESULTS AND DISCUSSION

A FINDINGS

The effects of gibberellins (GA4/GA7) on the nitrification of nitrogen derived from Lucerne meal are summarised in Table 9.5/01-1. In general, the concentration of inorganic nitrogen species (ammonium, nitrite and nitrate) increased over the course of the incubation, in both soils and at both concentrations of GA4/GA7. The increase in inorganic nitrogen formed at the expense of the organic nitrogen provided in the form of Lucerne meal indicates that the mineralisation processes remained functional, by virtue of the fact that all the mineralised nitrogen on Days 14 and 28 was in the form of nitrate, the terminal nitrification product, without accumulations of ammonium and/or nitrite. The impact of the GA4/GA7 treatments may therefore be assessed on the basis of the nitrate data alone. A threshold of 15% was applied to identify biologically significant differences from the solvent control.

Table 9.5/01-1 : Summary of the effects of 28-day exposure to gibberellins (GA4/GA7) on nitrogen mineralization by soil microflora

Gibberellins (GA4/GA7) nominal concentration (mg/kg dw soil)	Mean inorganic nitrogen concentration (mg N/100 g dry soil) *[% difference from mean control]*					
	Soil 1 (loamy sand)			Soil 2 (sandy loam)		
	Day 0	Day 14	Day 28	Day 0	Day 14	Day 28
ammonium (NH_4^+-N)						
Control	0.64	<LOQ	<LOQ	0.38	<LOQ	<LOQ
0.013	0.75	<LOQ	<LOQ	0.52	<LOQ	<LOQ
0.13	0.67	<LOQ	<LOQ	0.40	<LOQ	<LOQ
nitrite (NO_2^--N)						
Control	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
0.013	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
0.13	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
nitrite (NO_3^--N)						
Control	1.88	4.38	6.42	3.91	9.49	9.09
0.013	1.89 *[1]*	4.17 *[-5]*	5.89 *[-8]**	4.05 *[4]**	8.34 *[-12]*	8.89 *[-2]*
0.13	1.87 *[0]*	3.29 *[-25]**	5.56 [-13]*	3.87 [-1]	8.02 [-15]	8.91 [-2]

LOQ: Limit of quantitation. Analytical detection limits were 0.04, 0.12 and 0.30 mg N/100 g soil for NH_4^+-N, NO_2^--N and NO_3^--N, respectively.

* Statistically significantly different from the control ($p < 0.05$).

At the lower application rate (equivalent to 0.013 mg a.s./kg dw soil) effects on nitrate concentrations were consistently within ±15% of the mean control values for both soils throughout the incubation. At the higher application rate (equivalent to 0.13 mg a.s./kg dw soil) there was a transient reduction in nitrate concentrations ≥15% relative to the corresponding control in both soils on Day 14, but these differences were reduced to less than 15% in both soils by Day 28.

In the reference study, 125 mg dinoseb acetate/kg dry loamy sand soil caused concentrations of nitrate and total inorganic nitrogen to deviate from the unamended control by more than 15% at all measurement points, including Day 28.

III CONCLUSIONS

Application of gibberellins (GA4/GA7) at a concentration of 0.013 mg/kg dry soil had no biologically significant effect on the transformation of organic nitrogen in either of two natural field soils. Application at the higher concentration of 0.13 mg/kg dry soil initially suppressed the mineralisation sand subsequent nitrification of organic nitrogen by the indigenous microflora in both soils. This effect was transient and the differences between these processes in the presence and absence of the test substance had diminished to less than 15% within 28 days. Gibberellins (GA4/GA7) caused no permanent effect on nitrogen transformation in soils at the concentrations applied.

RMS comments and conclusion:

The study was conducted according to the test guideline ‘Guidelines for the official examination of plant protection products, Part VI, 1-1 (2. Edition), Effects on the activity of soil microflora (BBA, 1990)’. This guideline is no longer in use and has been replaced by test guideline ‘OECD 216 Soil microorganisms: nitrogen

transformation test'. The OECD 216 test guideline is designed to investigate the long-term effect of chemicals, after a single exposure, on nitrogen transformation activity of soil microorganisms.

Validity: According to the OECD 216 test guideline the following criteria should be satisfied in the controls for a test result to be considered valid:

- The variation between replicate control samples should be less than ± 15% **(in test: coefficient of variation for control samples is between 0.26% and 13.2%, condition fulfilled)**

The applicant has not provided information regarding variation of control samples. The RMS has calculated coefficients of variation based on data provided in the study report. Data were reported only as mean ± SD, no raw data were reported. This is not in line with test guideline that requires individual and mean data for nitrate measurement to be reported.

The study report states mean soil nitrogen concentrations, which is not in line with the test guideline that requires nitrogen formation rate to also be reported.

The applicant pointed out that in the case of Soil 2 (sandy loam), the control NO_3 concentration is lower (9.09) at D28 than at the preceding measurement on D14 (9.49). The applicant considers this to be an artefact due to analytical error. However, it gives rise to a negative formation rate for the control for the final 14-day interval: -0.03 mg NO_3^--N/100 g/d; compared to contemporary +0.04 and +0.06 mg NO_3^--N/100 g/d for 0.013 and 0.13 mg GA4/GA7/kg dw soil, respectively. The differences in nitrogen formation rate between GA4/GA7 treatment and control are in excess of the ±25% divergence permitted by OECD 216 test guideline. The RMS observed that in control as well as in GA4/GA7 treated samples nitrogen formation rate has reached a plateau at D14. This calls into question the study design and performance. The RMS considers the results in Soil2 to be unreliable.

The results of Soil1 (loamy sand) show no effect of gibberellins GA4/GA7 treatment on nitrogen formation rate. These results are considered reliable.

The sand content in the soils used in the study is not reported. It is an understanding of the RMS that the composition of loamy sand soil is typically: 70 to 90 % sand, 0 to 30 % silt and 0 to 15 % clay. The composition of sandy loam soil is typically: 60 % sand, 10 % clay and 30 % silt particles. Based on the reported characteristics of the two soils, neither is perfectly in line with the requirements of the OECD 216 test guideline. The test guideline requires test to be performed with one soil. The RMS will consider results obtained in Soil1 for risk assessment.

In this study the soil nitrogen concentrations were analysed on days 0, 14 and 28. This deviated from the test guideline which requires soil nitrogen concentrations to be analysed on days 0, 7, 14 and 28. The RMS considers this to be a minor deviation.

Despite several deviations from the OECD 216 test guideline the RMS considers the study to be adequate to show the absence of effects of gibberellins GA4/GA7 on soil microorganisms.

Stability of the test compound was not tested in this study.

Acceptability of the analytical methods used in the test: Not applicable.

Endpoints: Treatment with gibberellins GA4/GA7 at concentration 0.013 mg/kg dw soil had no effect on nitrogen transformation in loamy sand soil.

Conclusion of the RMS: The test is considered adequate to show the absence of effects of gibberellins GA4/GA7 on soil microorganisms.

b) Evaluation of additional data for the purpose of renewal of Annex I inclusion

No additional data was submitted or required for the purpose of renewal of approval of gibberellins GA4/GA7.

B.9.6. EFFECTS ON TERRESTRIAL NON-TARGET HIGHER PLANTS

B.9.6.1. Summary of screening data

a) Previous evaluation (2005-2011)

Under EU Reg. 283/2013 screening data shall not be used for active substances with plant growth regulatory activity. CA 8.6.2 applies. The summary of screening data was not submitted for the first inclusion of gibberellins GA4/GA7.

b) Evaluation of additional data for the purpose of renewal of Annex I inclusion

No additional data was submitted or required for the purpose of renewal of approval of gibberellins GA4/GA7.

B.9.6.2. Testing on non-target plants

a) Previous evaluation (2005-2011)

Toxicity test on non-target plants was not submitted for the first inclusion of gibberellins GA4/GA7. No data gap was identified in EFSA conclusions (EFSA Journal 2012;10(1):2502). Because gibberellins GA4/GA7 are naturally occurring substance it was considered that non-target plants will not be exposed to concentrations higher than naturally occurring levels when the products is used according to the proposed use pattern. Low acute or long-term risk was concluded on the basis of a qualitative argument concerning the non-toxic mode-of-action of gibberellins GA4/GA7 for the proposed use of gibberellic acid.

b) Evaluation of additional data for the purpose of renewal of Annex I inclusion

A study of the effects of gibberellins GA4/GA7 on the emergence and early development of seedlings of non-target terrestrial plant species has been performed with the technical active substance and is summarised below.

Data point addressed:	CA 8.6.2/01
Author(s) (year):	Stead, A. (2018)
Title:	Gibberellins A4A7: GLP Seedling Emergence and Seedling Growth Test Terrestrial Non-Target Plants (based on OECD Guideline 208) – 2017.
Laboratory report / project number:	STC/17/E1126
Testing facility:	Stockbridge Technology Centre Ltd, Selby, UK
Published:	No
Test guideline used:	OECD 208 (2006)
Deviations:	None
GLP:	Yes
Endpoint:	ER_{50} for all species tested >222 g a.s./ha (based on shoot fresh weight reduction)

Executive summary

A seedling emergence and seedling growth test was conducted for Gibberellins A4A7 applied pre-emergence to four monocotyledon species (onion, ryegrass, corn and wheat) and six dicotyledon species (oilseed rape, cabbage, soybean, tomato, lettuce and sugar beet). The test item was applied at six rates: 6.938, 13.875, 27.75, 55.5, 111 and 222 g test item/ha at a water application volume of 200 L/ha. Isopropyl alcohol (IPA) was used to aid the solubilisation of the test item at the highest treatment rate. Additionally, a water control and an IPA solvent control (40 mL IPA in 5 L water) were tested. For all plant species and test item rates tested, 94-100% of

seeds emerged (assessed 21 days after 50% emergence in controls). Of those that emerged, 100% of plants survived at all test item rates (assessed 21 days after 50% emergence in controls) for all species, with the exception of lettuce with lowest survival of 72% at 222 g test item/ha. Slight visual injury (such as elongated, thinner stems) was observed in ryegrass, tomato, lettuce and sugar beet. Shoot fresh weight (measured at 21 days after 50% emergence in controls) relative to the water control ranged from 74.2% (in lettuce at 222 g test item/ha) to 120.1% (in ryegrass at 111 g test item/ha). However, there were no statistically significant differences in shoot fresh weight reduction relative to the control at the highest rate tested, resulting in a No-Observed-Effect-Rate (NOER) based on shoot weight reduction of 222 g a.s./ha for all species tested. The ER_{50} based on shoot fresh weight reduction was >222 g a.s./ha for all species tested.

I MATERIALS AND METHODS

A MATERIALS

Test item:	Gibberellins A4A7
Lot No.:	1000048922
Purity:	90.16% (w/w)
Expiry date:	July 2019
Description:	White powder
Stability of test compound:	Not reported. Test substance is expected to have been adequately stable for the purposes of this study.

Test species and cultivar:
Allium cepa (onion) Hypark
Lolium perenne (ryegrass) Twystar
Zea mays (corn) LG 30179
Triticum aestivum (wheat) J. B. Diego
Brassica napus (Oilseed rape) Vision
Brassica oleracea (Cabbage) Sherwood
Glycine max (Soybean) Siverka
Lycopersicon esculentum (Tomato) Shirley
Lactuca sativa (Lettuce) Osterley
Beta vulgaris (Sugar beet) Haydn

Soil characteristics

Soil mix:	Sand 72.96%, Silt 16.63%, Clay 10.41%
Grade of sand content:	Very fine sand 5.11%, Fine sand 15.46%, Medium sand 31.88%, Coarse sand 20.15%, Very coarse sand 0.37%, Stones >2mm 39.00%
Texture:	Sandy loam
pH:	8.0
Organic matter DUMAS:	1.4%
Organic carbon:	0.8%
Electrical conductivity:	2240.00 µS/cm

Test conditions

Temperature:	16.9 to 25.9°C
Light intensity:	≥5000 lux, photosynthetically active radiation (PAR) 304 to 828 µmol/m^2/sec
Photoperiod:	16 h, natural daylight supplemented with artificial lighting

B STUDY DESIGN

The trial was of a randomised block design with five replicates of each treatment for each species. Each species was treated as a separate trial to ensure optimal watering. For all test species, 10 seeds were sown per pot (replicate). Seeds of all species were sown into 16 cm non-porous plastic pots containing a synthetic sandy loam soil mix (sharp sand: pasteurised loam: grit at ratio by volume of 4:2:2). The quantity of synthetic sandy loam soil mix added to each pot was approximately 1.8 L.

One day after sowing at pre-crop emergence), test item applications were conducted in a covered storage area. The test item was applied at six rates: 6.938, 13.875, 27.75, 55.5, 111 and 222 g test item/ha at a water application volume of 200 L/ha. Isopropyl alcohol (IPA) was used to aid the solubilisation of the test item at the highest treatment rate. Additionally, a water control and an IPA solvent control (40 mL IPA in 5 L water) were tested. All treatments were applied using a gas pressurised Oxford Precision Sprayer with a 2 m boom fitted with 4 fan tip 80º standard nozzles (ISO size: 01F80) mounted on a battery powered track sprayer. The track sprayer was calibrated after selecting the appropriate nozzles to achieve the required spray characteristic. Beakers were placed under each nozzle and the output collected for 30 seconds. The forward speed was then adjusted to achieve the required spray volume of 200 L/ha (± 10%). The test item treatments were applied as follows:

1. 5.55 g of Gibberellins A4A7 was added to 40 mL IPA and sonicated until all had dissolved. This solution was added to 5 L of mains tap water and sonicated for a further 1.5 hours until all test item had dissolved. Pots requiring the highest treatment rate (222 g test item /ha) were sprayed with the primary solution.
2. 2.5 L of the remaining primary spray solution was diluted with 2.5 L mains tap water and pots requiring the next treatment rate were sprayed.
3. This serial dilution process was repeated until all treatment rates had been applied.

Immediately following application, two 200 mL samples of the primary spray solution (Gibberellins A4A7 at 222 g test item/ha) were decanted from the tank mix and stored in the freezer prior to analysis to determine the active substance concentration. The sample was analysed using an external standard high-performance liquid chromatography technique.

Immediately prior to treatment application, all species except lettuce were lightly watered overhead with a watering can. Lettuce was watered immediately after sowing. Following treatment application, all pots were again lightly watered overhead with a hosepipe and each pot was placed on a plastic saucer. All subsequent watering was done by sub-irrigation to avoid leaching of the chemical from the surface of the soil. Plants were inspected on a daily basis and watered with mains tap water according to the individual crop requirement. The final watering was applied one or two days before the harvest of each species.

Powdered fertiliser was added to the synthetic sandy loam soil mix placed in the pots into which the onion, oilseed rape, cabbage, soybean, tomato, lettuce and sugar beet seeds were sown (0.5 g of 14:16:18 nitrogen:phosphorus:potassium per litre synthetic sandy loam soil mix). Oilseed rape and sugar beet also received liquid fertiliser feed three weeks after application (powdered fertiliser (18:11:18 N:P:K) was diluted to give a feed solution containing 1 g fertiliser/L water).

Untreated control plants were observed to determine when 50% of control plants had emerged. 14 and 21 days after 50% of the untreated plants had emerged, all plants were assessed for emergence, mortality or signs of visual injury. At 21 days after 50% of the untreated plants had emerged, all live plants were harvested and weighed to determine shoot fresh weight.

II RESULTS AND DISCUSSION

A FINDINGS

The measured concentration of Gibberellins A4/7 in the primary spray solution was 822.4 mg a.s./L against a nominal concentration of 1001 mg a.s./L, representing a recovery of 82%.

A summary of the mean highest percentage emergence (as a percentage of the number sown) is presented in the table below for all species tested.

Table 9.6.2/01- 1 : Summary of % seed emergence following exposure to GA4/GA7

Treatment GA4/GA7 (g test item/ha)	% emergence (21 days after 50% emergence in controls)									
	Onion	**Rye grass**	**Corn**	**Wheat**	**OSR**	**Cab bage**	**Soy bean**	**Tom ato**	**Let tuce**	**Sugar beet**
Water control	100	100	100	100	100	100	100	100	100	100
IPA control	100	100	100	100	100	100	100	100	98	100
6.938	98	100	100	100	100	100	94	100	96	98
13.875	100	100	98	100	100	100	100	100	98	100
27.75	100	100	100	100	100	100	100	100	98	100
55.5	100	100	100	100	100	100	100	100	98	100
111	100	100	100	96	100	100	98	100	98	100
222	100	98	100	100	100	100	100	100	100	98

A summary of the mean survival of plants at harvest (21 days after 50% emergence in controls) as a percentage of the highest number emerged is presented in the table below for all species tested.

Table 9.6.2/01- 2 : Summary of % survival following exposure to GA4/GA7

Treatment GA4/GA7 (g test item/ha)	% survival (21 days after 50% emergence in controls) as % of highest number emerged									
	Onion	**Rye grass**	**Corn**	**Wheat**	**OSR**	**Cab bage**	**Soy bean**	**Tom ato**	**Let tuce**	**Sugar beet**
Water control	100	100	100	100	100	100	100	100	100	100
IPA control	100	100	100	100	100	100	100	100	100	100
6.938	100	100	100	100	100	100	100	100	100	100
13.875	100	100	100	100	100	100	100	100	98	100
27.75	100	100	100	100	100	100	100	100	86	100
55.5	100	100	100	100	100	100	100	100	90	100
111	100	100	100	100	100	100	100	100	90	100
222	100	100	100	100	100	100	100	100	72	100

Onion, corn, wheat, oilseed rape, cabbage and soybean did not display signs of visual injury at any treatment rate tested. For ryegrass, very slight twisting of leaves was observed at rates ≥27.75 g test item/ha. Slight visual injury was also observed for tomato at rates ≥55.5 g test item/ha, consisting of elongated, thinner stems with some curling to the leaves. Slight to moderate visual injury on lettuce was observed at rates ≥13.875 g test item/ha, consisting of elongated, thinner, twisted stems with plants leaning over. For sugar beet, very slightly longer, thinner stems were observed at the highest rate tested; 222 g test item/ha.

A summary of the mean total shoot fresh weight at harvest (21 days after 50% emergence in controls) is presented in the table below for all species tested.

Table 9.6.2/01- 3 : Mean total shoot fresh weight at harvest following exposure to GA4/GA7

Treatment GA4/GA7 (g test item/ha)	Mean total shoot fresh weight at harvest (21 days after 50% emergence in controls) (g)									
	Onion	Rye grass	Corn	Wheat	OSR	Cab bage	Soy bean	Tom ato	Let tuce	Sugar beet
Water control	2.40	1.61	26.13	5.51	18.99	21.79	24.32	16.87	6.85	16.98
IPA control	2.60	1.68	25.84	5.06	17.23	20.95	24.79	16.86	5.69	15.78
6.938	2.64	1.46	25.86	4.87	16.56	19.64	21.72	15.37	6.64	16.44
13.875	2.62	1.69	27.54	5.68	18.71	20.71	24.01	15.21	5.71	17.88
27.75	2.70	1.67	26.56	5.31	19.73	19.39	23.97	15.31	5.44	15.45
55.5	2.83	1.64	26.88	5.25	16.69	20.27	23.83	16.81	6.24	18.31
111	2.49	1.94	27.05	5.23	17.39	20.03	25.67	15.88	5.74	18.98
222	2.50	1.77	27.66	5.41	18.43	18.63	25.17	12.80	5.08	16.84

A summary of the mean total shoot fresh weight at harvest (21 days after 50% emergence in controls) expressed as a percentage of the water control is presented in the table below for all species tested.

Table 9.6.2/01- 4 : Mean total shoot fresh weight at harvest as a percentage of the water control following exposure to GA4/GA7

Treatment GA4/GA7 (g test item/ha)	Mean total shoot fresh weight at harvest (21 days after 50% emergence in controls) (% of the water control)									
	Onion	Rye grass	Corn	Wheat	OSR	Cab bage	Soy bean	Tom ato	Let tuce	Sugar beet
Water control	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
IPA control	108.4	103.8	98.9	91.8	90.7	96.1	101.9	99.9	83.0	92.9
6.938	109.9	90.7	99.0	88.5	87.2	90.2	89.3	91.1	96.8	96.8
13.875	109.3	104.6	105.4	103.2	98.5	95.1	98.7	90.2	83.3	105.3
27.75	112.4	103.5	101.7	96.4	103.9	89.0	98.6	90.7	79.3	90.9
55.5	117.8	101.9	102.9	95.4	87.9	93.0	98.0	99.6	91.0	107.8
111	103.7	120.1	103.5	94.9	91.6	91.9	105.6	94.1	83.8	111.8
222	104.2	109.5	105.9	98.1	97.0	85.5	103.5	75.9	74.2	99.2

The ER_{10}, ER_{25}, ER_{50} and NOER values based on reduction in shoot fresh weight are summarized in the table below for all species tested.

Table 9.6.2/01- 5 : ER_{10}, ER_{25} and ER_{50} values based on reduction in shoot fresh weight following exposure to GA4/GA7

Species	ER_{10} (g test item/ha)	ER_{25} (g test item/ha)	ER_{50} (g test item/ha)	NOER (g test item/ha)
Onion	>222	>222	>222	222
Ryegrass	>222	>222	>222	222
Corn	>222	>222	>222	222
Wheat	>222	>222	>222	222
Oilseed rape	>222	>222	>222	222
Cabbage	<6.938	>222	>222	222
Soybean	>222	>222	>222	222
Tomato	<6.938	>222	>222	222
Lettuce	121.64	>222	>222	222
Sugar beet	>222	>222	>222	222

B VALIDITY

The test was considered valid as the following validity criteria were met:

- Seedling emergence in the controls was at least 70% (100%) for all species tested.
- Control seedlings did not exhibit visible phytotoxic effects (e.g. chlorosis, necrosis, and wilting, leaf and stem deformation) and only exhibited normal variation in growth and morphology for that particular species.
- Mean survival of emerged control seedlings was at least 90% (100%) for the duration of the study for all species tested.
- Environmental conditions for a particular species were identical and growing media contained the same amount of soil matrix, support media or substrate from the same source.

III CONCLUSIONS

A seedling emergence and seedling growth test was conducted with Gibberellins A4A7 applied pre-emergence to four monocotyledon species (onion, ryegrass, corn and wheat) and six dicotyledon species (oilseed rape, cabbage, soybean, tomato, lettuce and sugar beet). For all plant species and test item rates tested, 94-100% of seeds emerged (assessed 21 days after 50% emergence in controls). Of those that emerged, 100% of plants survived at all test item rates (assessed 21 days after 50% emergence in controls) for all species, with the exception of lettuce with lowest survival of 72% at 222 g test item/ha. Slight visual injury (such as elongated, thinner stems) was observed in ryegrass, tomato, lettuce and sugar beet. Shoot fresh weight (measured at 21 days after 50% emergence in controls) relative to the water control ranged from 74.2% (in lettuce at 222 g test item/ha) to 120.1% (in ryegrass at 111 g test item/ha). However, there were no statistically significant differences in shoot fresh weight reduction relative to the control at the highest rate tested, resulting in a No-Observed-Effect-Rate (NOER) based on shoot weight reduction of 222 g a.s./ha for all species tested. The ER_{50} based on shoot fresh weight reduction was >222 g a.s./ha for all species tested.

RMS comments and conclusion:

The OECD 208 Terrestrial Plant Seedling emergence and seedling growth test is designed to assess effects on seedling emergence and early growth of higher plants following exposure to the test substance in the soil 14 and 21 days after 50% emergence of the seedlings in the control group.

Validity: According to the OECD 208 test guideline the following criteria should be satisfied in the controls for a test result to be considered valid:

- Seedling emergence is at least 70% **(in test: seedling emergence for water control and IPA control = 100%, condition fulfilled)**
- The seedling do not exhibit visible phytotoxic effects (e.g. chlorosis, necrosis, wilting, leaf and stem deformations) and the plants exhibit only normal variation in growth and morphology for that particular species **(in test: controls exhibited no phytotoxic effects, condition fulfilled)**
- The mean survival of emerged control seedlings is at least 90%for the duration of the study **(survival for water control and IPA control = 100%, condition fulfilled)**
- Environmental conditions for particular species are identical and growing media contain the same amount of soil matrix, support media, or substrate from the same source **(condition fulfilled)**

Humidity during the study was in the range 24% to 99%. This is outside of the recommended range 70 ± 5% during light periods and 90 ± 5% during dark periods, as stated in Annex 4 of OECD 208 test guideline. The applicant provided the following explanation for this deviation: 'Moisture is dependent on the number of plants in the glasshouse and the amount of watering they receive which is a situation that can vary over time. As the plants are watered by putting water into saucers, the relative humidity can also be affected by when they are watered and can potentially decrease before being re-watered. Consequently, on some occasions, relative humidity can be slightly below or slightly above 70% (±25%). However, for this study this was not detriment of the plants as photographs of the untreated plants taken at harvest show.' The RMS is of the opinion that it cannot be excluded that such high variations in humidity had an effect on the test organisms. To exclude the effects of humidity on growth it would be useful to have historical control data for seedling emergence and growth of tested plant species. However, the seedling emergence and survival of water control and IPA control are at 100% and the photographs of the plants show that plants are in good conditions. The RMS considers the results of the study to be reliable.

Acceptability of the analytical methods used in the test: The analytical method for the determination of GA4/7 in the spray solution is considered valid and acceptable according to SANCO/3029/99 rev.4. No further data required. The assessment is reported in Section B.5.1.2.6.

Endpoints: The NOER based on shoot weight reduction of 222 g a.s./ha for all species tested. The ER_{50} based on shoot fresh weight reduction was >222 g a.s./ha for all species tested.

Conclusion of the RMS: The test is considered valid.

A study of the effects of gibberellins GA4/GA7 on the vegetative vigour of non-target terrestrial plants (Fiebig, 2017) has been performed with the representative product and a summary is presented at DRAR Vol.3 CP Novagib B.9.11.2.

B.9.7. EFFECTS ON OTHER TERRESTRIAL ORGANISMS (FLORA AND FAUNA)

a) Previous evaluation (2005-2011)

No other groups of terrestrial organisms are considered to be at risk and no concerns were raised from a review of the open literature. No other special areas of concern or data gaps were identified during the evaluation of gibberellins GA4/GA7 for Annex I inclusion. No further data available.

b) Evaluation of additional data for the purpose of renewal of Annex I inclusion

No additional data was submitted or required for the purpose of renewal of approval of gibberellins GA4/GA7.

B.9.8. EFFECTS ON BIOLOGICAL METHODS FOR SEWAGE TREATMENT

a) Previous evaluation (2005-2011)

An activated sludge respiration inhibition test for gibberellins GA4/GA7 was submitted and evaluated during the Annex I inclusion of GA4/GA7 and is available in the EU DAR. This study was considered acceptable in the EFSA conclusion for the risk assessment of effects on biological methods for sewage treatment. This study is considered appropriate to address the effects on biological methods for sewage treatment for the purposes of renewal. Full details of the study are provided in the EU DAR and related documents and references are listed in DRAR Vol.2. No new studies are submitted.

PREVIOUS EVALUATION	This study was evaluated in the original DAR and has been considered by EFSA. No new evaluation has been performed. The conclusion has not been changed.
Data point addressed:	CA 8.8/01 (II A 8.7/01 in original DAR)
Author(s) (year):	Barnes, S.P. (2004)
Title:	Gibberellins A4 and A7 (technical grade) Activated sludge- respiration inhibition test
Laboratory report / project number:	ZAB 048/042941
Testing facility:	Huntingdon Life Sciences Ltd., Cambridgeshire, UK
Published:	No
Test guideline used:	OECD 209; EC 88/302
Deviations:	None
GLP:	Yes
EU Agreed Endpoint:	3 h EC_{50} > 100 mg a.s./L

Executive summary

The effect of gibberellin (GA4/GA7) on the respiration of activated sludge was determined in a 3-hour test. The 3-hour EC_{50} is > 100 mg/L (nominal), the highest concentration tested. The NOEC is considered to be 100 mg a.s./L.

I MATERIALS AND METHODS

MATERIALS

Test material:	Gibberellins A4 + A7 (technical Grade)
Lot/Batch No.:	107-554-CD
Purity:	90.3% Total Gibberellins A4 + A7 (Technical Grade)
Stability of test compound:	Not reported, but may be considered to be adequately stable under the conditions of this study based on the behaviour observed in other tests.

Test organism:	Mixed population of micro-organisms indigenous to a sample of activated sludge.
Source:	Worlingworth STP, which treats predominantly domestic sewage. The activated sludge sample was collected on the day before use and maintained under aerobic conditions.
Treatments:	Four groups: control and gibberellin at nominal concentrations of 1, 10 and 100 mg/L. Additionally, the reference inhibitor 3,5-dichlorophenol (3,5-DCP) was tested in parallel at nominal concentrations of 3, 10 and 32 mg/L.
Replication	Two (control) and three (100 mg a.s./L) replicates, all other test and reference treatments were tested singly.
Sludge concentration:	1.6 g dry suspended solids/L in all incubation mixtures.
Duration:	3 hours.
Conditions:	The test vessels were incubated in a thermostatically-controlled water bath and their contents (total volume 500 mL) were continually aerated with a supply of oil-free compressed air delivered at a rate of 1 L/min. All mixtures contained an equal volume (16 mL) of OECD synthetic sewage that provided an organic respiration substrate.
Temperature:	19.7 to 21.2°C.
Photoperiod:	Not relevant.
Confirmatory analysis:	None.
Observations:	Measurements of oxygen consumption (respiration) rates were made sequentially in portions of each test mixture at the end of the 3-h incubation under conditions of forced aeration. Measurements ran for a period of *ca*. 10 minutes or until the dissolved oxygen concentration fell to below 2 mg/L and rates were determined from linear portions of the recorded data. pH and temperature were monitored at the start and end of the test.
Statistical analysis:	Not required for GA4/GA7. The EC_{50} and corresponding 95% confidence limits of the reference substance were calculated by the Moving average method using the computer program of Stephan (1977 and 1982).

II RESULTS AND DISCUSSION

A FINDINGS

The mean specific respiration rate of the control replicates after 3 h was 21.2 mg O_2/g/h. Inhibition relative to the mean control ranged from 0% to 6% in the GA4/GA7 treatments and is too low to be considered biologically significant.

Respiration in the reference mixtures was suppressed by 24%, 59% and 86% at 3, 10 and 32 mg 3,5-DCP/L, respectively. The 3 h EC_{50} was determined to be 7.5 mg 3,5-DCP/L with 95% confidence limits of 6.0 and 9.3 mg/L.

B VALIDITY

The EC_{50} for the reference toxicant lay within the acceptable range of 5 to 30 mg 3,5-DCP/L and the variation in specific respiration rate between the control replicates was within the acceptable limit of 15%.

III CONCLUSIONS

The effect of gibberellin (GA4/GA7) on the respiration of activated sludge was determined in a 3-hour test. The 3-hour EC_{50} is > 100 mg/L (nominal), the highest concentration tested. The NOEC is considered to be 100 mg a.s./L.

RMS comments and conclusion:

The OECD 209 Activated Sludge Respiration Inhibition Test aims to determine the effects of a substance on micro-organisms from activated sludge (largely bacteria) by measuring their respiration rate (carbon and/or ammonium oxidation) under defined conditions in the presence of different concentrations of the test substance.

Validity: According to the OECD 209 test guideline the Activated Sludge Respiration Inhibition test is considered acceptable if the following validity criteria are met:

- The blank controls (without the test substance or reference substance) oxygen uptake rate should not be less than 20 mg oxygen per one gram of activated sludge (dry weight of suspended solids) in an hour **(in test: 21.2 mgO_2/g/h, condition fulfilled)**

- The coefficient of variation of oxygen uptake rate in control replicates should not be more than 30% at the end of definitive test **(in test: 6%, condition fulfilled)**

- The EC_{50} of 3,5-DCP has to lie in the range 2 mg/L to 25 mg/L for total respiration, 5 mg/L to 40 mg/L for heterotrophic respiration and 0.1 mg/L to 10 mg/L for nitrification respiration **(in test: 3h EC_{50} = 7.5mg 3,5-DCP/L, condition fulfilled)**

The heterotrophic respiration and nitrification respiration are not reported. The OECD 209 test guideline states that the measurement of total oxygen uptake inhibition should be adequate. The RMS considers this a minor deviation that does not affect the validity of the result.

The pH during the study was 7.5– 8.1; this is slightly outside the required range 7 – 8. The RMS considers this to be a minor deviation that does not affect the validity of the result.

Stability of the test compound was not tested in this study.

Acceptability of the analytical methods used in the test: Not applicable.

Endpoints: The 3-hour EC_{50} is > 100 mg/L (nominal), the NOEC is 100 mg a.s./L.

Conclusion of the RMS: The activated sludge respiration inhibition test is considered valid.

b) Evaluation of additional data for the purpose of renewal of Annex I inclusion

No additional data was submitted or required for the purpose of renewal of approval of gibberellins GA4/GA7.

B.9.9. MONITORING DATA

a) Previous evaluation (2005-2011)

No further data available. Monitoring is likely to be futile as it will not be possible to discriminate between GA4/GA7 applied as a PPP and natural background sources. As additional information, the ubiquitous presence of GA4/GA7 and other gibberellins in the environment, arising from natural sources in plants, fungi and bacteria, is outlined in the published review summary below (CA 8.9/01) and referenced in DRAR Vol.2.

Data point addressed:	CA 8.9/01
Author(s) (year):	MacMillan, J. (2002)
Title:	Occurrence of Gibberellins in Vascular Plants, Fungi, and Bacteria
Laboratory report / project number:	Published. Journal of Plant Growth Regulation, 20, 387-442
Testing facility:	Department of Agricultural Sciences, University of Bristol, UK
Published:	Yes
Test guideline used:	N/A
Deviations:	N/A
GLP:	No

Executive summary

This review paper provides a compilation of the occurrence of GAs (GA_1 to GA_{126}) in vascular plants, fungi and bacteria, together with the type of tissue in which the identification was made. This study summary lists only the findings presented for GA3, GA4 and GA7.

I MATERIALS AND METHODS

The natural occurrence of GAs in plants was initially established by their isolation, mainly from immature seeds in which the amounts are high. Since the early 1970s their identification from natural sources has relied mainly upon combined gas chromatography-mass spectrometry (GC-MS) data from purified, derivatized extracts and a comparison with reference GC-MS data. In this review paper the following criteria for the identification of the GAs have been applied:

1. Only publications in refereed journals are cited

2. Identification from normal (tall) phenotypes is given preference

3. Preliminary communications are not cited where a full paper has been published

4. GAs identified after hydrolysis of plant extracts are not included

5. Some papers, even though they are contained in refereed journals, have not been cited on the grounds that they are judged to contain insufficient data upon which to justify claimed identification.

II RESULTS AND DISCUSSION

The data presented in this review paper for GA_1 to GA_{126} show the widespread natural occurrence of GAs (128 plants, 7 fungi, and 7 bacteria). Summaries of the natural occurrence of GA3, GA4 and GA7 are presented in the tables below.

The results show that GA3 has been found in 45 plant species, across 15 different families, often in immature or mature seeds, as well as in roots, shoots, leaves, fruits, pollen and silk. GA3 has also been found in 1 fungus and 6 bacteria species.

The results show that GA4 has been found in 54 plant species, across 29 different families, including in seeds, leaves, shoots, buds, fruits and pollen. GA4 has also been found in 7 fungi and 3 bacteria species.

The results show that GA7 has been found in 14 plant species, across 9 different families, including in seeds, leaves, shoots and pollen, as well as in 1 fungus species.

A FINDINGS

Table 9.9/01- 1 : List of tissues of vascular plants, fungi and bacteria in which GA3 has been found

Plant	Tissue	Common name of plant	Plant family
Abelmoschus esculentus	immature seed	Okra	Malvaceae
Althaea rosea	shoot apices	Common hollyhock	Malvaceae
Arabidopsis thaliana	seeds	Thale cress	Brassicaceae
Avena sativa	inflorescences	Oat	Poaceae
Brassica napus	stems, immature siliques	Oilseed rape	Brassicaceae
Calystegia soldanella	immature seeds	Bindweed	Convolvulaceae
Camellia sinensis	endosperm	Tea plant	Theaceae
Carica papaya	fruits	Papaya	Caricaceae
Carthamus tinctorius	stems	Safflower	Asteraceae
Citrus sinensis	fruitlets	Blood orange	Rutaceae
Cucumis melo	mature seeds	Muskmelon	Cucurbitaceae
Cucumis sativus	mature seeds	Cucumber	Cucurbitaceae
Dalbergia dolichopetela	germinating seed		
Fragaria x ananassa Duch.	immature fruit- day neutral, leaves- day neutral, leaf exudates- short day	Strawberry	Rosaceae
Hordeum vulgare	germinating grain, leaf sheaths, developing grain, shoots	Barley	Poaceae
Ipomoea batatas	immature seeds	Sweet potato	Convolvulaceae
Ipomoea reptans	immature seeds	Water spinach	Convolvulaceae
Lactuca sativa	shoots	Lettuce	Asteraceae
Lolium temulentum	leaves	Darnel	Poaceae
Lycopersicon esculentum	cultured roots, leaves and shoot tips, unpollinated ovaries	Tomato	Solanaceae
Lupinus albus	seeds	Lupin	Fabaceae
Malus domestica	immature seeds	Apple	Rosaceae
Mangifera indica	leaves	Mango	Anacardiaceae
Marah macrocarpus	endosperm, embryo	Wild cucumber	Cucurbitaceae
Pennisetum glaucum	shoots	Millet	Poaceae
Pharbitis purpurea	immature seeds	Common morning glory	Convolvulaceae
Pharbitis tricolor	immature seeds	Mexican morning glory	Convolvulaceae
Phaseolus coccineus	immature seeds	Runner bean	Fabaceae
Phaseolus lunatus	stems, root nodules	Butter bean	Fabaceae
Picea abies	shoots	Norway spruce	Pinaceae

Plant	Tissue	Common name of plant	Plant family
Picea sitchensis	shoots	Sitka spruce	Pinaceae
Pinus attenuata	pollen	Knobcone pine	Pinaceae
Pinus sylvestris	stem and needles	Scots pine	Pinaceae
Pisum sativum	pods, ovules, pollinated ovaries	Pea	Fabaceae
Prunus avium	fruitlets, germinating seeds, apices ex 10-wk and mature plants	Wild cherry	Rosaceae
Prunus cerasus	immature seeds	Sour cherry	Rosaceae
Prunus persica	immature seeds	Peach	Rosaceae
Pseudotsuga menziesii	shoots	Douglas fir	Pinaceae
Saccarum spp	leaves, shoot apical meristem	Sugarcane	Poaceae
Secale cereale	plants	Rye	Poaceae
Sechium edule	endosperm, embryo, testa	Chayote	Cucurbitaceae
Triticum aestivum	leaves, roots, stems, shoots, expanding internodes, young ears	Common wheat	Poaceae
Vigna unguiculata/ sinensis	leaves, petioles, epicotyls, stems	Cowpea	Fabaceae
Vitis vinifera	seeds, seeded berries	Common grapevine	Vitaceae
Zea mays	shoots, silk	Maize	Poaceae
Fungi			
Gibberella fujikuroi			
Bacteria			
Acetobacter diazotropicus			
Azospirillum lipoferum			
Azospirillum brasilense			
Bacillus licheniformis			
Bacillus pumilus			
Herbospirillum seropedicae			

Table 9.9/01- 2 : List of tissues of vascular plants, fungi and bacteria in which GA4 has been found

Plant	Tissue	Common name of plant	Plant family
Abelmoschus esculentus	immature seed	Okra	Malvaceae
Allium cepa	leaf sheaths	Onion	Amaryllidaceae
Alstroemeria hybrid	leaves	Lily	Alstroemeriaceae
Anemia phyllitidis	sporophytes	Fern	Anemiaceae
Arabidopsis thaliana	shoots, seeds	Thale cress	Brassicaceae
Aralia cordata	basal buds	Spikenard	Araliaceae
Begonia x cheimantha	leaves	Christmas begonia	Begoniaceae
Brassica napus	immature siliques	Oilseed rape	Brassicaceae
Calystegia soldanella	immature seeds	Bindweed	Convolvulaceae
Cibotium glaucum	sporophytes	Tree fern	Cibotiaceae

Plant	Tissue	Common name of plant	Plant family
Citrus reticulata	developing fruit	Mandarin orange	Rutaceae
Citrus sinensis	immature fruit	Blood orange	Rutaceae
Citrus unshiu	young fruit, developing fruit	Tangarine	Rutaceae
Cucumis melo	mature seeds	Muskmelon	Cucurbitaceae
Cucumis sativus	mature seeds	Cucumber	Cucurbitaceae
Cucurbita maxima	endosperm, embryo	Squash	Cucurbitaceae
Cyathea australis	sporophytes	Rough tree fern	Cyatheaceae
Dalbergia dolichopetela	germinating seed		
Daucus carota	somatic cell embryo cultures	Wild carrot	Apiaceae
Dicksonia antarctica	sporophytes	Soft tree fern	Dicksoniaceae
Dioscorea opposita	dormant bulbils	Yam	Dioscoreaceae
Eucalyptus globulus	cambial region	Tasmanian bluegum	Myrtaceae
Helianthus annuus	seeds	Sunflower	Asteraceae
Hordeum vulgare	developing grain	Barley	Poaceae
Juglans regia	pollinated and unpollinated ovaries	Common walnut	Juglandaceae
Lilium elegans	bulbs	Lily	Liliaceae
Lupinus albus	seeds	Lupin	Fabaceae
Lycopersicon esculentum	leaves and shoot tips	Tomato	Solanaceae
Malus domestica	immature seeds, developing seeds	Apple	Rosaceae
Marah macrocarpus	endosperm, embryo	Wild cucumber	Cucurbitaceae
Matthiola incana	shoots and flower buds	Stock	Brassicaceae
Ornithogalum thyroides	infloresecences	Chinkerinchee	Asparagaceae
Orobanche minor	arial parts	Common broomrape	Orobanchaceae
Oryza sativa	ears, spikelets	Rice	Poaceae
Phaseolus coccineus	dark-grown seedlings, light-grown seedlings, cotyledonary embryo, suspensor	Runner bean	Fabaceae
Phaseolus vulgaris	immature seeds	Common bean	Fabaceae
Picea abies	shoots	Norway spruce	Pinaceae
Picea sitchensis	shoots	Sitka spruce	Pinaceae
Pimpinella anisum	somatic cell embryo cultures	Aniseed	Apiaceae
Pinus attenuata	pollen	Knobcone pine	Pinaceae
Pinus sylvestris	stem and needles	Scots pine	Pinaceae
Pisum sativum	internodes, fertilized ovules	Pea	Fabaceae
Pseudotsuga menziesii	shoots	Douglas fir	Pinaceae
Raphanus sativus	leaves, stem	Radish	Brassicaceae
Rumex acetosa	shoots	Sorrel	Polygonaceae
Rumex palustris	shoots	Marsh dock	Polygonaceae
Saccharum spp	apical meristem	Sugarcane	Poaceae

Plant	Tissue	Common name of plant	Plant family
Sechium edule	endosperm, embryo	Chayote	Cucurbitaceae
Spinacia olerocea	shoots	Spinach	Amaranthaceae
Trifolium repens	arial parts	White clover	Fabaceae
Triticum aestivum	leaves and stems, expanding internode, shoots	Common wheat	Poaceae
Vigna unguiculata/ sinensis	hypocotyls	Cowpea	Fabaceae
Vitis vinifera x V.rupestris	somatic embryos	Grapevine	Vitaceae
Zea mays	shoots	Maize	Poaceae
Fungi			
Gibberella fujikuroi			
Phaeospheria sp. L489			
Sphaceloma bidentis			
Sphaceloma manihiticola			
Sphaceloma menthea			
Sphaceloma perseae			
Sphaceloma rhois			
Bacteria			
Bacillus licheniformis			
Bacillus pumilus			
Rhizobium phaseoli			

Table 9.9/01- 3 : List of tissues of vascular plants, fungi and bacteria in which GA7 has been found

Plant	Tissue	Common name of plant	Plant family
Calystigia soldanella	seeds	Bindweed	Convolvulaceae
Daucus carota	somatic cell embryo cultures	Wild carrot	Apiaceae
Malus domestica	immature seeds, developing seeds	Apple	Rosaceae
Marah macrocarpus	endosperm, embryos	Wild cucumber	Cucurbitaceae
Ornithogalum thyroides	inflorescences	Chinkerinchee	Asparagaceae
Picea abies	shoots	Norway spruce	Pinaceae
Pimpinella anisum	somatic cell embryo cultures	Aniseed	Apiaceae
Pinus attenuata	pollen	Knobcone pine	Pinaceae
Pisum sativum	fertilized ovules	Pea	Fabaceae
Pseudotsuga menziesii	shoots	Douglas fir	Pinaceae
Sechium edule	endosperm, embryos, testa	Chayote	Cucurbitaceae
Spinacia oleracea	shoots	Spinach	Amaranthaceae
Triticum aestivum	leaves and stems	Common wheat	Poaceae
Zea mays	shoots	Maize	Poaceae
Fungi			
Gibberella fujikuroi			
Bacteria			
None listed			

III CONCLUSIONS

The data presented in this review paper for GA_1 to GA_{126} show the widespread natural occurrence of GAs in plants, fungi and bacteria (reported here in 128 plants, 7 fungi, and 7 bacteria).

GA3 has been found in 45 plant species, across 15 different families, often in immature or mature seeds, as well as in roots, shoots, leaves, fruits, pollen and silk. GA3 has also been found in 1 fungus and 6 bacteria species.

GA4 has been found in 54 plant species, across 29 different families, including in seeds, leaves, shoots, buds, fruits and pollen. GA4 has also been found in 7 fungi and 3 bacteria species.

GA7 has been found in 14 plant species, across 9 different families, including in seeds, leaves, shoots and pollen, as well as in 1 fungus species.

RMS comments and conclusion:

The RMS agrees that the study is sufficient to show widespread natural occurrence of gibberellins GA4/GA7.

b) Evaluation of additional data for the purpose of renewal of Annex I inclusion

No additional data was submitted or required for the purpose of renewal of approval of gibberellins GA4/GA7.

B.9.10. BIOLOGICAL ACTIVITY OF METABOLITES POTENTIALLY OCCURRING IN GROUNDWATER

There are no ecotoxicologically relevant metabolites of gibberellins GA4/GA7 occurring in groundwater (see DRAR Vol 3 CA B.8.6).

B.9.11. REFERENCES RELIED ON

Summary of the public literature search

Two searches were undertaken for relevant literature in the public domain on the active substance gibberellin (GA47 and relevant synonyms). The initial search was undertaken in April 2016, and a supplementary search was also carried out in November 2017 following the 1-year extension to the submission deadline for the renewal dossier.

Both searches were conducted in accordance with:

- Commission Implementing Regulation (EU) No 844/2012, as referred in Article 8(5) of Regulation (EC) No 1107/2009 and,

- the EFSA document; Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) No 1107/2009. EFSA Journal 2011; 9(2) : 2092.

The search strategies for both were based on a single concept search. For details regarding the search strategy, relevance criteria applied and the results obtained, please see the Literature Review Reports (KCA 9 & KCP 11).

The selection process in each search resulted in three categories of publication:

- Publications which meet the relevance criteria and are assessed to be reliable which are addressed at the appropriate data points in the relevant MCA & MCP Sections of the dossier.

- Publications which meet the relevance criteria but are assessed to be non-reliable are referenced and a justification for not meeting the reliability criteria provided in Section 6 of this Literature Review Report.

- Publications not meeting the relevance criteria are referenced in Section 6 of this Literature Review Report.

Results of Initial Search (April & May 2016)

In the April 2016 search 1,157 summary records were retrieved from bibliographic databases and were screened by expert reviewers and grouped into two categories according to their likely relevance after rapid assessment of titles and, when available, abstracts:

1. Obviously not relevant: 1,126 summary records.

   These summary records (titles and/or abstracts) did not contain specific information relevant to the criteria specified in Table 1 of the KCA 9 report.

2. Not excluded after rapid assessment: 31 summary records were classified as potentially relevant and thus were assessed in detail, a full assessment of the full-text documents.

3. Following assessment 29 of the full text documents were excluded from the dossier.
4. Following assessment 2 of the full text documents were considered to be of interest but as these were EFSA Conclusions they are not specifically listed as references in the dossier.

After discussion with the applicant, it was decided that the next phase of searching should use more specific Residues and Toxicology/Human Health nested search terms only.

In the updated search (May 2016), 418 summary records were retrieved from bibliographic databases and were screened by expert reviewers and grouped into two categories according to their likely relevance after rapid assessment of titles and, when available, abstracts:

1. Obviously not relevant: 399 summary records.

These summary records (titles and/or abstracts) did not contain specific information relevant to the criteria specified in Table 1 of the KCA 9 report.

2. Not excluded after rapid assessment: 19 summary records were classified as potentially relevant and thus were assessed in detail, a full assessment of the full-text documents.

3. Following assessment 19 of the full text documents were excluded from the dossier.

4. Following assessment 0 of the full text documents were considered to be of interest.

It was concluded that 0 of the 418 summary records were relevant.

Results of Top Up Search (November 2017)

In summary, in the November 2017 search 1,728 summary records were retrieved from bibliographic databases and were screened by expert reviewers and grouped into two categories according to their likely relevance after rapid assessment of titles and, when available, abstracts:

1. Obviously not relevant: 1,695 summary records.

These summary records (titles and/or abstracts) did not contain specific information relevant to the criteria specified in Table 1.

2. Not excluded after rapid assessment: 33 summary records were classified as potentially relevant and thus were assessed in detail, a full assessment of the full-text documents.

3. Following assessment 31 of the full text documents were excluded from the dossier.

4. Following assessment 2 of the full text documents were relevant.

It was concluded that 2 of the 1,728 summary records were relevant. Full details can be found in the Literature Review Reports (KCA 9 & KCP 11) and the relevant papers are included in dossier under the appropriate the KCA & KCP data points.

RMS comments and conclusion:

The RMS confirms that the literature search was performed and is considered adequate. Detailed public literature review was reported in 2 documents provided by the applicant. The search process is documented in all details according the GUIDANCE OF EFSA, Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) No 1107/2009, EFSA Journal 2011;9(2):2092. For the section of ecotoxicology no studies of relevance were identified.

Data Point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
CA 8.1.1.1/01	[redacted]	1996	Falgro Technical: An acute oral toxicity study with the Northern Bobwhite Report No. [redacted] GLP Unpublished	Y	N	-	Fine Agrochemicals Ltd	In DAR II A 8.1.1/01
CA 8.1.1.1/03	[redacted]	1991	Gibberellic acid Acute Oral Toxicity (LD_{50}) to Mallard Duck. Huntingdon Research Centre Ltd report No.: [redacted] GLP, unpublished.	Y	N	-	Valent Biosciences	In DAR II A 8.1.1/02
CA 8.1.1.2/01	[redacted]	1996	Falgro Technical: A dietary LC_{50} study with the Northern Bobwhite Report [redacted] GLP Unpublished	Y	N	-	Fine Agrochemicals Ltd	In DAR II A 8.1.2/01
CA 8.2.1/01	[redacted]	2004a	Gibberellins A_4 & A_7 – Acute toxicity to rainbow trout (*Oncorhynchus mykiss*) under static-renewal conditions Report No. [redacted] GLP Unpublished	Y	N	-	VBC	In DAR II A 8.2.1/01
CA 8.2.2.1/01	[redacted]	2016	Gibberellic acid (GA3): An early life-stage toxicity test with the Fathead Minnow (*Pimephales promelas*) Report No. [redacted] GLP Unpublished	Y	Y	New study submitted for the purpose of renewal	VBC	N
CA 8.2.3,	Abdellaoui, K.	2013	Biochemical and histological effects of	N	N	-	-	N

CA 8.1.5			gibberellic acid on *Locusta migratoria migratoria* fifth instar larvae					
CA 8.2.3, CA 8.1.5, CA 5.8.3	Saito, K.	2008	Reporter gene assay for gibberellic acid (GA4/GA7) using human estrogen and androgen receptors	N	N	-	GA4/7 Task Force	N
CA 8.2.4.1/01	Sayers, L.E.	2004b	Gibberellins A4 & A7 – Acute toxicity to water fleas (*Daphnia magna*) under static conditions Report No. 13828.6102 Springborn Smithers Laboratories, Wareham, USA GLP Unpublished	N	N	-	VBC	In DAR II A 8.2.4/01
CA 8.2.5.1/01	Juckeland, D.	2014	Toxicity of gibberellins (GA4/GA7) technical to *Daphnia magna* in a 21-day semi-static reproduction test Report No. 14 10 48 073 W BioChem agrar, Gerichshain, Germany GLP Unpublished	N	Y	New study submitted for the purpose of renewal	Globachem	N
CA 8.2.6.1/01a	Gries, T.	2000	Gibberellins A4 + A7 – Static toxicity test with the freshwater algae *Pseudokirchneriella subcapitata* Report No. 1042.004.430 Springborn Laboratories (Europe) AG, Horn, Switzerland GLP Unpublished	N	N	-	VBC	In DAR II A 8.2.6/01
CA 8.2.6.1/01b	Collison, E.	2017	Re-assessment of validity of Gries (2000): Gibberellins A4 + A7 – Static toxicity test with the freshwater algae *Pseudokirchneriella subcapitata*; Report No. 1042.004.430. Springborn Laboratories (Europe) AG, Horn, Switzerland [KCA 8.2.6.1/01] Non-GLP Unpublished	N	Y	New study submitted for the purpose of renewal	GA4/GA7 TF	N
CA	Mantilacci, S.	2017	Toxicity evaluation of test item	N	Y	New study	GA4/GA7 TF	N

8.2.6.2/01			Gibberellins GA4/GA7 Technical on *Navicula pelliculosa* in a growth inhibition limit test and validation of the analytical method Report No. BT264/17 BioTecnologie B.T. Srl, Italy GLP Unpublished			submitted for the purpose of renewal		
CA 8.3.1.1/01	Hoberg, J.R.	2004a	Gibberellins A4 & A7 – Acute oral toxicity test with the honey bee (*Apis mellifera*) Report No. 13828.6103 Springborn Smithers Laboratories, Wareham, USA GLP Unpublished	N	N	-	VBC	In DAR II A 8.3.1.1/01
CA 8.3.1.1/02	Hoberg, J.R.	2004b	Gibberellins A4 & A7 – Acute contact toxicity test with the honey bee (*Apis mellifera*) Report No. 13828.6104 Springborn Smithers Laboratories, Wareham, USA GLP Unpublished	N	N	-	VBC	In DAR II A 8.3.1.1/02
CA 8.3.1.2/01	Gray, J.	2017	Gibberellins A_4A_7: Honey bees (*Apis mellifera* L.) chronic oral toxicity test 10 day feeding in the laboratory Report No. FR30QH Envigo CRS Ltd., Huntingdon, UK GLP Unpublished	N	Y	New study submitted for the purpose of renewal	GA4/GA7 TF	N
CA 8.3.1.3/01	Taylor, K.	2017	Gibberellins A_4A_7: Honey bee (*Apis mellifera*) larval toxicity test, single exposure Report No. CR15QN Envigo CRS Ltd., Huntingdon, UK GLP Unpublished	N	Y	New study submitted for the purpose of renewal	GA4/GA7 TF	N
CA 8.3.2/01	Nienstedt, K.M.	1999a	Gibberellin A4 & A7 – Toxicity test with	N	N	-	VBC	In DAR

			the parasitic wasp, *Aphidius rhopalosiphi* (Hymenoptera: Braconidae) Report No. 1042.008.270 Springborn Smithers Laboratories (Europe) AG, Horn, Switzerland GLP Unpublished					II A 8.3.2/01
CA 8.3.2/02	Nienstedt, K.M.	1999b	Gibberellin A4 & A7 – Laboratory contact toxicity test with the predacious mite, *Typhlodromus pyri* Scheuten (Acari: Phytoseiidae) Report No. 1042.008.268 Springborn Smithers Laboratories (Europe) AG, Horn, Switzerland GLP Unpublished	N	N	-	VBC	In DAR II A 8.3.2/02
CA 8.4.1/01	Porch, J.R. & Krueger, H.O.	2001	ABG-3192 [Gibberellin A4 + A7]: An acute toxicity study with the earthworm in an artificial soil substrate Report No. 529-103 Wildlife International Ltd., Maryland, USA GLP Unpublished	N	N	-	VBC	In DAR II A 8.4.1/01
CA 8.4.1/02	Sloman, T.L. and Porch, J.R.	2017	Gibberellic acid: a reproduction study with the earthworm in an artificial soil substrate Report No. 529P-109 EAG Laboratories, Maryland, USA GLP Unpublished	N	Y	New study submitted for the purpose of renewal	GA4/GA7 TF	N
CA 8.5/01	van der Kolk, J.	2000	Gibberellin A4 & A7 – The effects on the respiration and nitrification of soil microflora Report No. 1042.006.748 Springborn Smithers Laboratories (Europe) AG, Horn, Switzerland GLP Unpublished	N	N	-	VBC	In DAR II A 8.5/01
CA 8.6.2/ 01	Stead, A.	2018	Gibberellins A4A7: GLP Seedling	N	Y	New study	VBC	N

			Emergence and Seedling Growth Test Terrestrial Non-Target Plants (based on OECD Guideline 208) – 2017 Report No. STC/17/E1126 Stockbridge Technology Centre, Cawood, UK GLP Unpublished			submitted for the purpose of renewal		
CA 8.8/01	Barnes, S.P.	2004	Gibberellins A4 and A7 (technical grade) Activated sludge- respiration inhibition test Report No. ZAB 048/042941 Huntingdon Life Sciences Ltd., Cambridgeshire, UK GLP Unpublished	N	N	-	VBC	In DAR II A 8.8/01
CA 8.9/01	MacMillan, J.	2002	Occurrence of Gibberellins in Vascular Plants, Fungi, and Bacteria Journal of Plant Growth Regulation, 20, 387-442 Non-GLP Published	N	N	-	-	In DAR B.7.1.3
CA 5.8.3	Saito, K.	2008	Reporter gene assays for gibberellic acid (GA3) using human estrogen and androgen receptors Report No. TLT-0106 Environmental Health Science Laboratory, Sumitomo Chemical Co., Ltd, 1-98, 3-Chome, Kasugade-Naka, Konohana-Ku, Osaka, Japan Non-GLP Unpublished	N	N	-	GA4/7 Task Force	N

APPENDIX 1

The excel spreadsheet containing the evidence for assessment of endocrine disrupting properties of gibberellins GA4/GA7 is provided separately (Appendix E)