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.6 (AS)

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

Version History

When	What
2019/April	Initial DRAR

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B.6. TOXICOLOGY AND METABOLISM 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).
Gibberellin 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 Gibberellin 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. However, full details from each study have not been repeated in this DRAR - therefore this DRAR is not a "stand-alone document" and for full reference sometimes the reader needs to consult the DAR (2005-2011).

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.6.1. ABSORPTION, DISTRIBUTION, METABOLISM AND EXCRETION IN MAMMALS

B.6.1.1. Absorption, distribution, metabolism and excretion by oral route

a) Previous evaluation (2005-2011)

Data on absorption, distribution, metabolism and excretion (ADME) were submitted during the EU review of GA4/7 and are available in the EU DAR. The study below was considered acceptable in the EFSA conclusion and is considered adequate for supporting renewal of GA4/7; no new ADME studies are submitted.

PREVIOUS EVALUATION	This study was evaluated in the DAR and has been considered by EFSA.
Data point addressed:	CA 5.1.1/01 (B.6.1.1-1 DAR)
Author(s) (year):	[redacted] (2000)
Title:	Absorption, Distribution, Metabolism and Excretion of [^{14}C]-gibberellins GA_4GA_7 in rats
Laboratory report / project number:	[redacted]
Testing facility:	[redacted]
Published:	No
Test guideline used:	OECD 417 (1984); US EPA 870.7485 (1995)
Deviations:	None (the study was conducted to an earlier version of the current guideline however the methodology is acceptable)
GLP:	Yes
EU Agreed Endpoint:	Oral absorption 40% in females and 18% in males

Executive summary

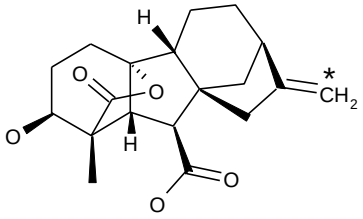
An ADME study was conducted in the rat with gibberellins GA4/7 (non-radiolabelled batch no 33263CD00, purity 90.8%; radiolabelled batch no 64794-GR-17-A-1, purity 98%, specific activity 57.2 mCi/mol), and included a single oral dose and a 15-day repeated oral dose. The low dose level was 65 mg/kg bw and the high dose level was 1000 mg/kg bw; the low dose was used for bile-duct cannulated rats. Absorption and excretion of gibberellins was found to be rapid, with wide distribution. Oral absorption was estimated at 40% in females and 18% in males based on urinary excretion at 48 hours, with no evidence for accumulation (98% of the recovered radioactivity was excreted via urine, faces and bile within 48 hours). Biliary excretion was the major route at low doses but a shift to urinary excretion occurred at high doses, particularly in females, implying a saturation of some part of the biliary route. Bile-cannulation experiments also demonstrated that at low doses the major part of the faecal radioactivity was excreted via the biliary route. The main metabolic pathway identified was hydroxylation and glucuronide conjugation of parent compound and hydroxyls; the proposed pathway is shown below.

I Materials and Methods

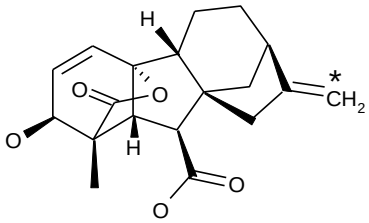
A. Materials:

1a. Test Material [^{14}C]-Gibberellin A4 and A7 (ratio between 70:30 and 75:25)

Description GA4 structure and [^{14}C] label position (*)



GA_7 structure and [^{14}C] label position (*)



Lot/Batch GA4: 64794-GR-17-A-1; GA7: 64794-GR-18-A-1

Purity GA4: 98%; GA7: 97%

1b. Test Material Non-radiolabelled Gibberellins GA4/7

Description Mixture of GA4 and GA_7 in ratio 72.5:27.5

Lot/Batch 33263CD00

Purity 90.8% (sum of GA4 and GA7)

2. Vehicle Isopropanol for dissolving radiolabelled materials, evaporated off prior to final formulation and final concentration prepared in 3% CMC

3. Test animals Rats

Species Sprague-Dawley Crl:CD:BR

Age Approximately 8 weeks old

Weight 175-300 g

Source [redacted]

Acclimation period 5 days including 1 day in metabolism cage

Diet Standard Lab rodent diet (Rat & Mouse No. 3 Breeding Diet), *ad libitum*

Water Tap water *ad libitum*

Housing Animals were housed individually in Plexiglass metabolism cages

4. Environmental conditions

Temperature 19-25°C

Humidity 30-70%

Air changes 10 air changes/hour

Photoperiod 12 hour light/dark cycle

B. Study Design and Methods

1. In life dates: 24 August 1999 to 15 May 2000

2. Animal assignment and treatment

Study design is detailed in Table CA 5.1.1-1. Five male and five female rats were allocated to Group B1 and dosed

at 50 mg [^{14}C] gibberellin A4 (equivalent to 65 mg/kg bw total gibberellins {A4 and A7}) in 3% CMC administered in a dose volume of 5 mL/kg bw. Samples were collected as follows-expired air at 0 - 24 and 24-48 hours post-dosing from one rat of each sex; blood at 0.5, 1, 2, 4, 6, 8, 12, 24, 48 and 96 hours; faeces over 0 to 24 hours and then at 24 hour intervals; urine over 0 - 8 and 8 - 24 hours then at 24 hour intervals. The cage wash was completed at the end of the sampling period. At termination 7 days after dosing, tissues and organs were examined for radioactivity and radioactivity in excreta, blood, organs and residual carcasses was analysed. Pooled urine and faeces samples were used for metabolite profiling.

Five male rats were allocated to Group B2 and dosed at 18 mg [^{14}C] gibberellin A_7 (equivalent to 65 mg/kg bw total gibberellins {A4 and A7}) in 3% CMC administered in a dose volume of 5 mL/kg bw. Samples were collected as described for Group B1.

Four male and four female rats were allocated to Group C and dosed at 50 mg [^{14}C] gibberellin A_4 (equivalent to 65 mg/kg bw total gibberellins {A4 and A7}) in 3% CMC administered in a dose volume of 5 mL/kg bw on Day 15. This was preceded by 14 daily oral doses of 1000 mg unlabelled gibberellins GA4A7/kg bw. The target radioactive dose was approximately 200 µCi/kg bw (7.4 MBq/kg bw). Samples were collected as follows-blood at 0.5, 1, 2, 4, 6, 8, 12, 24 and 48; faeces over 0-24 hours and then at 24 hour intervals; urine over 0-8 and 8-24 hours then at 24 hour intervals. The cage wash was completed at the end of the sampling period. At termination 7 days after dosing, tissues and organs (adrenals, brain, abdominal fat, heart, kidneys, liver, lungs, skeletal muscle, ovaries, prostate, residual carcass, abdominal skin, spleen, testes, uterus, plasma, whole blood) were examined for radioactivity and radioactivity in excreta, blood, organs and residual carcasses was analysed. Pooled urine and faeces samples were used for metabolite profiling.

Five male and five female rats were allocated to Group D and dosed at 700 mg [^{14}C] gibberellin A_4 (equivalent to 1000 mg/kg bw total gibberellins {A4 and A7}) in 3% CMC administered in a dose volume of 5 mL/kg bw on a single occasion. The target radioactive dose was approximately 200 µCi/kg bw (7.4 MBq/kg bw). Samples were collected as follows-expired air at 0-24 and 24-48 hours post dosing from one rat of each sex; blood at 0.5, 1, 2, 4, 6, 8, 12, 24, 48 and 96 hours; faeces over 0-24 hours and then at 24 hour intervals; urine over 0-8 and 8-24 hours then at 24 hour intervals. The cage wash was completed at the end of the sampling period. At termination 7 days after dosing, tissues and organs (adrenals, brain, abdominal fat, heart, kidneys, liver, lungs, skeletal muscle, ovaries, prostate, residual carcass, abdominal skin, spleen, testes, uterus, plasma, whole blood) were examined for radioactivity and radioactivity in excreta, blood, organs and residual carcasses was analysed. Pooled urine and faeces samples were used for metabolite profiling.

Twelve male and twelve female rats were allocated to Group Fb and dosed at 50 mg [^{14}C] gibberellin A_4 (equivalent to 65 mg/kg bw total gibberellins {A4 and A7}) in 3% CMC administered in a dose volume of 5 mL/kg bw on a single occasion. The target radioactive dose was approximately 100 µCi/kg bw (3.7 MBq/kg bw). Three rats of each sex were sacrificed at various timepoints, selected on the blood kinetic data obtained from Group B, at 1.5 hours (Tmax), 4 hr, 12 hours and 24 hr. Total urine, total faeces, cagewash and gastro-intestinal tract were analysed for radioactivity recovery. At termination tissues and organs as described above were examined for residual radioactivity and the radioactivity in excreta, organs and residual carcasses was analysed.

Twelve male and twelve female rats were allocated to Group Fd and dosed at 700 mg [^{14}C] gibberellin A4 (equivalent to 1000 mg/kg bw total gibberellins {A4 and A7}) in 3% CMC administered in a dose volume of 5 mL/kg bw on a single occasion. The target radioactive dose was approximately 100 µCi/kg bw (3.7 MBq/kg bw). Three male rats were sacrificed at 2 hours (Tmax), 8 hr, 12 hours and 24 hours and three females were each sacrificed at the timepoints 3 hours (Tmax), 6 hours, 12 hours and 24 hours. These termination points were selected using blood kinetics data from Group D. Total urine, total faeces, cagewash and gastro-intestinal tract were analysed for radioactivity recovery. At termination tissues and organs as described above were examined for residual radioactivity and the radioactivity in excreta, organs and residual carcasses was analysed.

Four male and four female bile-duct cannulated rats (with an average bile flow of at least 0.5 mL/h) were allocated to Group G and dosed at 50 mg [^{14}C] gibberellin A4 (equivalent to 65 mg/kg bw total gibberellins {A4 and A7}) in 3% CMC administered in a dose volume of 5 mL/kg bw on a single occasion. The target radioactive dose was

approximately 200 µCi/kg bw (7.4 MBq/kg bw). Samples were collected as follows-bile at 0-2, 2-4, 4-8, 8-12, 12-24 and 24-48 hours post dosing; faeces over 0-24 and 24-48 hours and urine over 0-8, 8-24 and 24-48 hours. The cage wash was completed at the end of the sampling period. At termination 48 hours after dosing, the bile, excreta GI tract with contents and residual carcass were analysed separately for remaining radioactivity. Pooled bile samples were analysed for metabolite profiling.

Dose solutions were prepared freshly immediately prior to dosing. Aliquots of each dosing solution were taken for analysis of purity and formulation stability. The radiochemical purity of each gibberellin (GA4 or GA7) was checked in the dose solution by HPLC. The purity for GA4 was >98% and approximately 94% for GA7. Further aliquots were removed before and after dosing to check for formulation homogeneity.

Analysis of liquid dose aliquot and liquid specimens from the various dose groups were analysed directly by liquid scintillation counting. Other samples were prepared by digestion or combustion and trapping $^{14}CO_2$ in liquid scintillant prior to LSC analysis. Limit of quantification was taken to be double the background count and to control bias all radio-assays were completed in duplicate, except for organs weighing <0.5g.

Metabolite profiling was completed by pooling samples/group, sex and time interval. Early bile samples were pooled by sex up to the 12 hour point. Urine samples were centrifuged and injected in to an HPLC system for analysis. Bile was diluted with Milli-Q water and then injected in to the HPLC. Faeces were homogenised in acetonitrile/formic acid, dried in a nitrogen stream and dissolved in Milli-Q water/acetonitrile. After centrifugation and aliquot was injected into the HPLC system for analysis.

3. Dose preparation

Dose formulations were prepared immediately prior to administration. Non-labelled test material was weighed into a vial and the required volume of radiolabelled test substance added. The isopropanol from the radiolabelled component was evaporated away and 3% CMC added to reach final volume/concentration. Dose solution aliquots were analysed for purity and stability of the formulation, the remainder was stored below -18°C. The radiochemical purity of ^{14}C-Gibberellin A_4 and A_7 was analysed using HPLC. GA_4 was >98% and GA_7 was approximately 94%. Dose formulation stability and homogeneity were checked by taking and analysing aliquots collected prior to and immediately after dose administration.

4. Statistics

Various calculations are detailed in the study report for determination of activity dosed/animal; radioactivity/gram of sample; radioactivity in µg parent compound equivalents/gram of tissue; radioactivity as percentage of administered dose and limit of quantification. First and terminal phase rate constants (k_{el}) were determined by linear regression of the concentration time curve and the elimination half-life calculated from K_{el}. AUC (0-t) was calculated by the linear trapezoidal rule and AUC (0-∞) by extrapolation.

Table B 6.1.1-1: Study design for ADME investigations with radiolabelled A4/A7

Dose group	Test material	Dose regimen	Dose levels mg/kg bw	Number of rats		Samples
				Males	Females	
B1* preliminary	GA4	Low oral	65	5	--	urine, faeces, expired air #, blood kinetics, tissues, carcass
B2* preliminary	GA7	Low oral	65	5	--	urine, faeces, expired air #, blood kinetics, tissues, carcass
B1	GA4	Low oral	65	--	5	urine, faeces, expired air #, blood kinetics, tissues, carcass
C	GA4	Repeat 14x high and 1x low oral	14 x 1000 1 x 65	4	4	urine, faeces, blood kinetics, tissues, carcass
D	GA4	High oral	1000	5	5	urine, faeces, expired air #, blood kinetics, tissues, carcass
Fb	GA4	Low oral	65	3 x 4	3 x 4	depletion kinetics in tissues
Fd	GA4	High oral	1000	3 x 4	3 x 4	depletion kinetics in tissues
G	GA4	Low oral	65	4	4	bile, urine, faeces, carcass, GI tract

 # from one rat/sex in each case; blood kinetic data obtained from remainder of group

II Results and Discussion

A. Blood kinetics

[^{14}C]-GA4 is rapidly absorbed from the gastrointestinal tract with a peak blood concentration at 1 or 2 hours post-dosing for the low and high dose levels respectively. The Cmax for the low dose was approximately 5 to 8 µg/g and for the high dose was 140 to 150 µg/g. Peak blood radioactivity levels were hardly affected by pre-treatment by 14 consecutive daily administrations of unlabelled gibberellins GA4/7. Radioactivity was rapidly absorbed and then progressively eliminated in a bi-phasic manner, showing both a distribution phase and an elimination phase. The increase in radioactivity concentration in blood at 8 hours post-dosing, particularly among the females, indicated enterohepatic circulation of GA4 or its metabolites.

B. Absorption

Absorption of [^{14}C]-GA4 after a low oral dose was greater than 90%. There was no notable difference in absorption between the sexes at the low dose level. Since no bile-duct cannulated high dose rats were included in the study design, the exact absorption of [^{14}C]-GA4 could not be determined. However, high dose absorption was calculated, based on percent radioactivity excreted in urine and retained in tissues, to be at least 43% in male rats and 78% in female rats.

C. Distribution

Radioactivity distribution was generally throughout the whole body but with high levels concentrated in organs of elimination (liver and kidneys). Within 24 hours tissue residues fell rapidly, in a bi-phasic manner, reducing by between 30 and 200 fold depending on sex and dose level, from the maximum levels at Tmax. In both sexes, tissue residues in all tissues except liver and kidneys, 24 hours after oral administration of low or high doses, were below 0.1 ppm and 1 ppm GA4 equivalents respectively. Tissue residue levels in rats pre-treated for 14 days with a high dose of non-radiolabelled test material followed by an intermediate dose of radiolabelled material (group C) were similar to levels in rats receiving a single dose (group B1) indicating that there was no accumulation following repeat dose administration. At 168 hours after administration of the low dose, the majority of tissue

residues (except liver and kidneys for females and liver and blood for males) were below the limit of quantification. At the high dose, tissue levels at 168 hours (where greater than LOQ) were higher, approximately in proportion to dose.

D. Excretion

At both low and high dose levels, more than 98% of recovered radioactivity was excreted via urine, faeces and bile within 48 hours of dosing and more than 80 % within 24 hours.

For the females, between 86.5 and 93.2% of the urinary radioactivity was present as unchanged GA4 at low and high doses; for the males, between 40.0 and 52.9% was unchanged GA4 in the low dose group, rising to 75.4% in the high dose group. Up to 76.0% of female faecal radioactivity was accounted for by unchanged parent GA4 and approximately 53% of male faecal radioactivity was present as unchanged GA4. Unchanged parent, GA4, represented 3.6% of administered dose in male bile and 5.2% in the female bile sample.

The bile-cannulation group demonstrated that the major part of the radioactivity in the faeces represented absorbed radioactivity that had been excreted via the biliary route. Urinary excretion was higher in females than males. At the high dose there was a shift from primarily biliary excretion (low dose) to urinary excretion for both males and females, implying that at higher doses there was a saturation of the biliary route. Pre-treatment for 14 days did not affect total excretion, and faecal excretion was only slightly retarded.

E. Metabolism

Urine: For the females, between 86.5 and 93.2% of the urinary radioactivity was present as unchanged GA4 at low and high doses; for the males, between 40.0 and 52.9% was unchanged GA4 in the low dose group, rising to 75.4% in the high dose group i.e. males appeared to metabolise the parent material to a greater extent than females. Pre-treatment for 14 days with unlabelled gibberellins GA4A7 had no effect on metabolite profiles for either sex. Fourteen metabolites were detected in urine. There was a marked difference in the metabolite profile for males and females at both low and high dose levels. Females also excreted a higher proportion of the administered radioactivity in the urine, compared to males. Two urinary metabolites were present in males in substantial quantities. U9 constituted 11.8% (low dose) and 6.5% (high dose) of the urinary radioactivity, equivalent to 1.3 and 2.8% of total administered radioactivity, respectively. U7 was present at 10.0% of low dose urinary radioactivity, equivalent to 1.1% of the administered dose. All other metabolites were present in all groups but at less than 5% of urinary radioactivity and <1% of total administered dose. There was no significant difference between the metabolic profiles of GA4 and GA7.

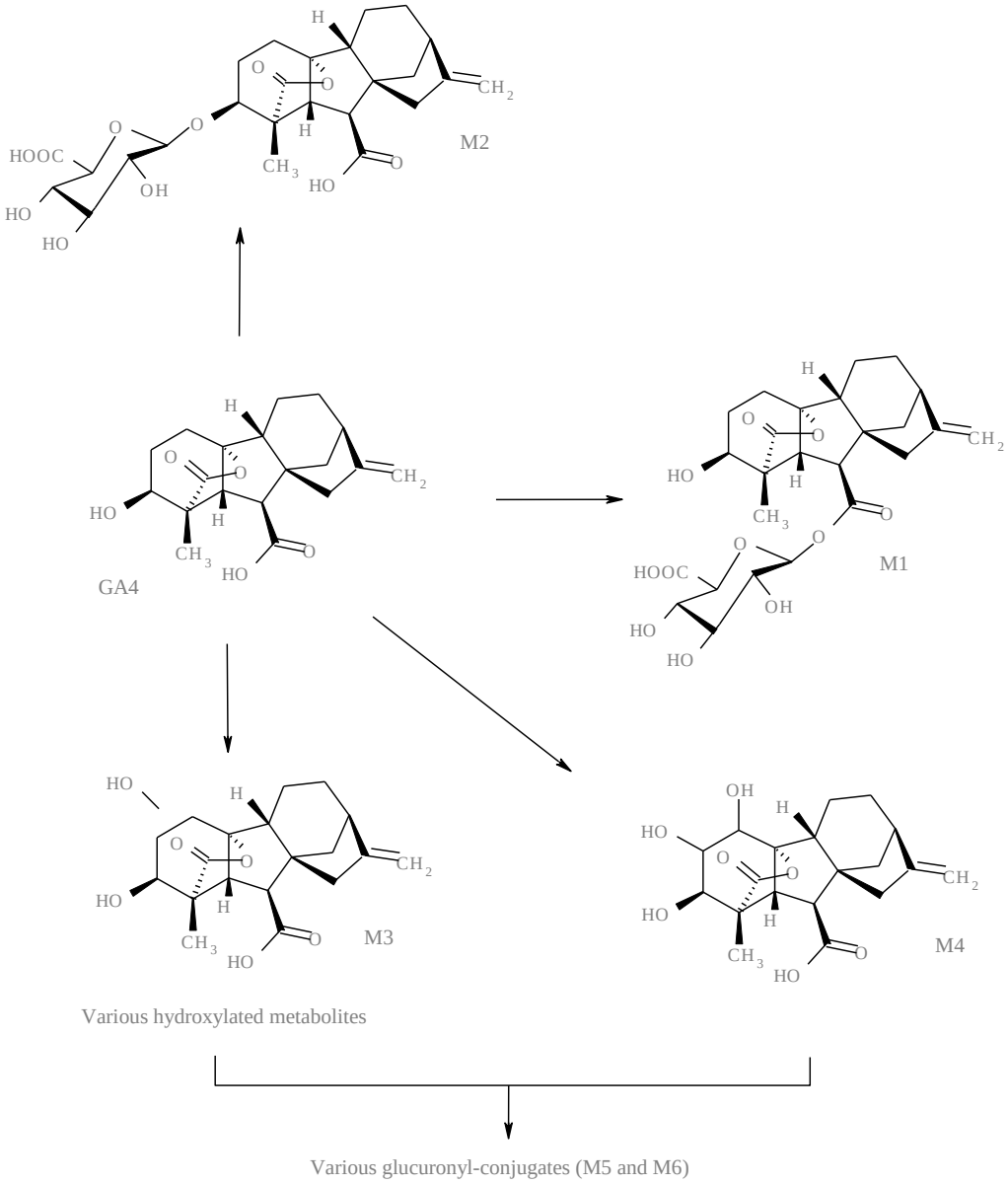
Faeces: Metabolite profiles for males and female faeces differed in a similar manner to the pattern observed in urine, with up to 76.0% of female faecal radioactivity accounted for by GA4 but only a maximum of 53.6% present as GA4 in males. Fourteen metabolites were detected in faeces together with the parent compound. There were significant dose-dependent differences in faecal metabolite profile for both sexes. In the low dose group, the most prominent metabolite for females was F11 (3.4% of administered dose) but for males it was F3 (14.1% of administered dose). In the high dose group, F11 was the most prominent metabolite in both females (11.3% of administered dose) and males (14.9% of administered dose). Results for the bile showed the low dose group for both sexes contained relatively high amounts of F11. It was therefore concluded that metabolite F11 was subject to reabsorption from the GI tract at low doses and underwent enterohepatic recirculation. At high doses it is presumed that reabsorption sites for F11 are saturated and consequently higher amounts appear directly in the faeces. Pre-treatment with unlabelled gibberellins GA4A7 at a low dose had little effect on the metabolite profile, with F3 remaining the major metabolite in males (16.3% of the dose). F3 was present at a lower level in the pre-treated females (0.3% of administered dose), however levels of all metabolites were lower in this treatment group. There was no significant difference between the metabolic profiles of GA4 and GA7.

Bile: Ten metabolites and parent compound were detected in bile. The major metabolite for both males and females was B11, representing 36.3 or 34% respectively, of cumulative dose over the 0-12 hour period. The second most important metabolite was B10, representing *ca* 10% of the administered dose in both sexes. A third major metabolite, representing 6.8% of the dose, was found in male bile but was almost completely absent from

female bile. Unchanged parent, GA4, represented 3.6% of administered dose in males and 5.2% in females. All other peaks in the metabolite profiles represented less than 3% of administered dose, in both sexes, and were not further identified.

Identification: When urine was incubated with ß-glucuronidase, two glucuronyl metabolites were detected. Metabolite U9 disappeared (1.3% of dose in male urine prior to glucuronidase treatment), the peak for metabolite U7 (1.1% of administered dose) decreased and the peak for metabolite U3 increased. In bile there were three glucuronyl metabolites detected. Metabolite B10 (representing approximately 10% of dose in both sexes) was shown to be the glucuronic acid metabolite of GA4. Metabolite B7 (6.8% of dose in males) and B9 (2.2% of dose in males) both had reduced peak height following glucuronidase incubation. No sulphate metabolites were detected in urine or bile. Metabolite B7 and B11 in bile were considered to be probably the same as faecal metabolites F7 and F11 based on retention times. The two metabolites, F7 and B7, occurred in the low dose at levels $\geq$ 5% as glucuronic acid conjugates. U3 and B2 were both thought to be di-hydroxylated metabolites. The numbered B and F metabolites showed the same retention times in the HPLC system, and were considered to be the same. The metabolite M1 is hypothetical, but the metabolite M2 was considered equivalent to B10, the glucuronide conjugate of GA4, and the dihydroxylated metabolite M4 may be F3, and F7 (=B7) appears to be the glucuronide conjugate of F3. While the exact identities of the metabolites cannot be determined, the data are sufficient to demonstrate adequately that metabolism is predominantly restricted to mono- and di-hydroxyls, and glucuronide conjugates of parent and hydroxyls.

Figure B 6.1.1-1 Proposed metabolic pathway of gibberellins GA4/7 in rats



Conclusion

EFSA (2012) estimated that oral absorption is 40% in females and 18% in males based on urinary excretion within 48 hours. There was no evidence for accumulation (98% of the recovered radioactivity was excreted via urine, faces and bile within 48 hours). The endpoint was agreed in the EFSA Conclusions and is still considered valid.

RMS comments and conclusion:

The ADME study was submitted during the previous EU review of GA4/7. The studies followed the OECD 417 (1984) and GLP principles. The study design complies with the latest version of the guideline OECD 417 (2010).

Rats were dosed with a single dose of 1000 mg/kg or 65 mg/kg of GA4. Continuous exposure was studied by dosing the rats for 14 day with “non-radioactive” 1000 mg/kg GA4 followed by the final dosing with radioactive

GA4 (equivalent to 65 mg/kg GA4). Pharmacokinetic behaviour of GA4 and GA7 was compared at the low dose of [14C]-GA4 and [14C]-GA7. No significant differences in blood kinetic and ADME parameters were observed thus, further studies were carried out using [14C]-GA4 as GA4 compared to GA7 represents a higher proportion in the GA4/GA7 technical material. Radioactive recovery was adequate.

From Table B.6.1.1-2 it can be seen that GA4 is rapidly absorbed from the GIT with T max of 1 hour and 32 hours post dose for the low and the high dose, respectively. C_{max} was not influenced by the 14 -days continuous dosing with the high dose. Radioactivity was progressively eliminated from the body showing a distribution and an elimination phase (see $t_{1/2}$). Enterohepatic circulation of GA4 which was also assumed. Radioactivity in the blood was increased in a dose-related manner in high dosed group showing high bioavailability of GA4 and a saturated elimination in the first phase.

Table B.6.1.1-2: Kinetic parameters for the groups treated with the low and high dose of [^{14}C]-GA_4 (B1 and D) and with the low dose of [^{14}C]-GA_7 (group B2), (from DAR 2011)

Parameter	B1 GA_4 low dose		B2 GA_7 low dose	C GA_4 repeated dose		D GA_4 high dose	
	Male	Female	Male	Male	Female	Male	Female
C_{max} (µg/g)	5.3	7.9	4.1	6.1	9.8	154	141
T_{max} (h)	1	1	1	1	1	2	2
$T_{1/2}$ (h) First phase	1.8	1.1	1.6	1.9	0.8	nd	nd
$T_{1/2}$ (h) Second phase	5	6	5	5	6	4	4
AUC_{0-24h}	25.7	28.8	18.9	33.8	24.5	1213	1644
$AUC_{0-\infty}$	26.5	29.8	19.3	35.1	35.3	1223	1648

nd: not determined

The excretion was rapid in all teste groups, competed within 48 hours. In the groups dosed with single low dose and multiple high doses with the final low dose the excretion occurred mainly via faeces in both sexes where biliary excretion represented the major part. Excretion via urine was higher in females than males. Total excretion in group C was not affected, faecal excretion was only slightly retarded. The shift from faecal to urinary excretion at the high dose could be possibly related to the saturation of the biliary route. First pass metabolism cannot be excluded.

Table B.6.1.1-3a: Excretion balance for 168 hours postdose for the low (B1, B2), high (D), repeated (C) dose groups and for 48 hours for the bile-duct cannulated (G) group (from DAR 2011)

Group	Sex	Percent of total radioactivity (%)					
		Faeces	Urine	Bile	Cage wash	**Total**	Retained (Carcass/Tissues)
Low dose (B1)	Male	97.2	11.0	-	0.2	**108.4**	0.04
Low dose (B1)	Female	60.1	41.6	-	0.8	**102.5**	0.11
Low dose (B2)	Male	82.4	16.0	-	0.1	**98.5**	0.05

Repeated dose (C)	Male	91.5	14.0	-	0.1	**105.6**	0.04
Repeated dose (C)	Female	61.0	45.5	-	0.4	**106.8**	0.11
High dose (D)	Male	61.0	43.1	-	0.3	**104.4**	0.06
High dose (D)	Female	28.2	78.2	-	0.6	**107.1**	0.15
Bile low dose (G)	Male	13.8	18.0	72.7	0.7	**105.3**	0.70
Bile low dose (G)	Female	8.2	39.4	55.7	0.4	**103.7**	0.31

Table B.6.1.1-3b Excretion balance for 168 hours postdose for the low (B1, B2), high (D), repeated (C) dose groups and for 48 hours for the bile-duct cannulated (G) group

Group		B1	B1	B2	C	C	D	D	G	G
Sex		M	F	M	M	F	M	F	M	F
Dose mg/kg		46.6	46.4	18.9	48.6	51	721	703	47.2	48.1
Urine	0- 24 h	10.1	39.2	15.4	13.1	42.6	42.4	76.1	17.7	38.8
	24- 48 h	0.9	1.6	0.4	0.7	2.2	0.5	1.4	0.3	0.6
	48-168 h	0.1	0.8	0.1	0.2	0.7	0.2	0.8	-	-
	Subtotal	**11.0**	**41.6**	**16.0**	**14.0**	**45.5**	**43.1**	**78.2**	**18.0**	**39.4**
Faeces	0- 24 h	88.6	53.4	77.4	67.1	46.3	57.1	22.8	12.2	2.5
	24- 48 h	8.3	6.1	4.7	22.5	13.4	3.7	4.8	1.6	5.7
	48-168 h	0.3	0.6	0.3	1.9	1.2	0.2	0.7	-	-
	Subtotal	**97.2**	**60.1**	**82.4**	**91.5**	**61**	**61**	**28.2**	**13.8**	**8.2**
Bile	0-24 h	-	-	-	-	-	-	-	72.3	54.9
	24-48 h	-	-	-	-	-	-	-	0.4	0.8
	Subtotal	-	-	-	-	-	-	-	**72.7**	**55.7**
Expired air*		<0.01	<0.01	< 0.01	-	-	<0.01	<0.01	-	-
Cage Wash		0.2	0.8	0.1	0.1	0.4	0.3	0.6	0.7	0.4
Total excretion		**108.4**	**102.5**	**98.5**	**105.6**	**106.8**	**104.4**	**107.1**	**105.3**	**103.7**
Tissue residues										
GI-tract		-	-	-	-	-	-	-	0.23	0.25
Organs		0.01	< 0.01	< 0.01	0.01	< 0.01	0.01	< 0.01	-	-
Tissue and blood		< 0.01	< 0.01	0.01	< 0.01	< 0.01	0.01	< 0.01	-	-
Residual carcass		0.03	0.11	0.03	0.03	0.11	0.04	0.14	0.48	0.06
Total Retained		**0.04**	**0.11**	**0.05**	**0.04**	**0.11**	**0.06**	**0.15**	**0.70**	**0.31**

- : not applicable

* :expired air measured in one animal per group.

The first sampling for tissue distribution was done around T_{max}, thus representing the highest dose of GA4 that tissue/organs are exposed to. GA4 was widely distributed in all organs. At the low dose, the highest levels were

found in liver and kidney in both sexes. At high dose the main radioactivity was found in kidney first, followed by liver and plasma. At 168 hours after administration of the low dose, the majority of tissue residues (except liver and kidneys for females and liver and blood for males) were below the limit of quantification. At the high dose, all tissue levels at 168 hours were above the limit of quantification. The tissue distribution data and excretion data show that bile excretion is considered an important excretion route which can saturate. The tissue distribution and route of excretion correlate well to the target organs identified in the short-term studies (liver and kidney), and in the reproductive toxicity study (kidney). First pass effect cannot be excluded, thus the predicted oral absorption in females is 40% and in males 18%.

The tissue distribution of GA4 coincides with the target organs (kidney and liver) observed the short-term studies.

Table B.6.1.1-4: Tissue distribution in low (Fb) and high (Fd) dose groups of rats (from DAR 2011)

Group	**Fb male**				**Fb female**			
Time point (hours post dosing)	**1.5h**	**4h**	**12h**	**24h**	**1.5h**	**4h**	**12h**	**24h**
Mean residues expressed as µg PCE/g tissue								
Whole Blood	6.50	1.98	0.38	0.085	7.34	1.52	0.45	0.055
Plasma	7.64	2.61	0.49	0.095	6.64	1.87	0.58	0.073
Liver	48.80	18.86	7.89	1.277	41.98	20.65	10.02	1.336
Kidneys	16.65	6.60	1.13	0.265	28.75	5.79	2.45	0.280
Lungs	3.04	0.93	0.16	0.047	2.62	0.68	0.23	0.030
Heart	4.97	1.64	0.28	0.061	5.46	1.22	0.35	0.042
Spleen	2.30	0.68	0.15	0.046	1.78	0.42	0.15	<LQ
Adrenals	2.74	0.72	<LQ	<LQ	2.86	0.45	0.31	<LQ
Brain	0.25	0.10	0.02	<LQ	0.22	0.08	0.02	<LQ
Testes/Uterus	1.52	0.81	0.16	0.033	1.60	0.48	0.22	0.073
Prostate/Ovaries	2.17	0.94	0.18	0.044	2.31	0.67	0.09	<LQ
Muscle	1.74	0.57	0.11	0.025	1.86	0.54	0.13	0.018
Skin	2.76	0.94	0.17	0.047	2.53	0.50	0.17	0.027
Fat	0.30	0.12	0.04	<LQ	0.31	0.12	0.06	<LQ
Carcass	2.92	1.20	0.23	0.082	3.23	1.24	0.76	0.101
Group	**Fd male**				**Fd female**			
Timepoint (hours post dosing)	**2h**	**8h**	**12h**	**24h**	**3h**	**6h**	**12h**	**24h**
Whole Blood	201.3	92.7	19.4	2.0	209.3	72.7	4.9	0.9
Plasma	253.1	126.9	26.0	0.7	267.8	99.5	7.1	1.2
Liver	351.8	236.2	84.9	7.7	271.9	176.3	42.6	17.7
Kidneys	462.8	197.1	62.3	3.6	514.3	268.5	21.9	4.7
Lungs	109.2	53.4	11.0	0.8	113.0	44.9	3.4	0.9
Heart	143.7	62.1	13.5	0.7	147.7	52.8	4.4	0.8
Spleen	85.7	37.3	8.3	1.0	86.8	28.1	2.1	0.5
Adrenals	95.8	38.5	8.9	<LQ	99.4	30.0	2.3	<LQ
Brain	9.7	5.1	1.6	<LQ	9.2	4.6	0.7	=LQ
Testes/Uterus	57.0	45.2	13.6	1.4	99.3	27.8	3.4	1.0
Prostate/Ovaries	74.7	31.6	8.3	1.1	107.0	43.3	3.1	<LQ
Muscle	80.3	30.1	6.6	0.6	78.0	24.4	2.2	0.4
Skin	86.3	25.9	10.2	1.0	94.1	64.5	2.7	1.2
Fat	9.9	2.6	2.5	<LQ	10.4	7.6	0.5	0.6
Carcass	107.3	52.6	11.8	2.5	104.9	34.9	5.4	12.5

LQ limit of quantification.

Dose and sex depended differences were observed in the metabolite profiles. Low levels of metabolites were detected in urine (below 3%) of both sexes in all groups. The major part represented the unchanged GA4. The major metabolite was U9 (high dose, males, 2.8%, low doses, males 1.3%) and the second one was the U7 (low and high dose, males, 1.1%). The unchanged GA4 represented also the major part of the detected metabolites in faeces of low dose and repeating dose groups; at the high dose, the percentage of unchanged GA4 was lower. The major metabolite in faeces was F11 (high dose, males 14.9%, females 11.3%) which was found equivalent to bile metabolite B11 (males 36.3%, females 34.0). For F11, enterohepatic circulation was hypothesized. Metabolites that were detected at doses ≥ 5% are shown with a red circle (Table 6.1.1-5). To detect metabolite conjugates with glucuronic acid and sulphate, the samples were treated with β-glucuronidase and arylsulphatase/β-glucuroidase. No sulphate-conjugated metabolites were detected. The identified glucuronide metabolites were: U7, U9, B7, B9, B10. U7 and U9 are supposed to be glucuronide conjugates of U3; B7 and B9 glucuronide conjugates of B2 and B10 a glucuronide conjugate of unchanged GA4. B7 and B11 are supposed to be the same metabolites as F7 and F11. B11 as a major metabolite was not affected by treatment thus probably not being a glucuronide conjugate. B2 and U3 are believed to be di-hydroxilated meatbolites and thus similar to the di-hydroxilated metabolite F3. The amounts B7 and B9 in bile are similar to those of F7 and F9 in faeces, which could indicate that this glucuronide conjugates are not cleaved in the intestine.

The identification of metabolites according to paragraph 42 of OECD417 (2010) has not been carried out (only HPLC analyses were performed with no additional spectroscopic analyses). From the data provided it can be deducted that metabolism is predominantly restricted to hydroxylation and glucuronide conjugation of parent compounds and hydroxyls.

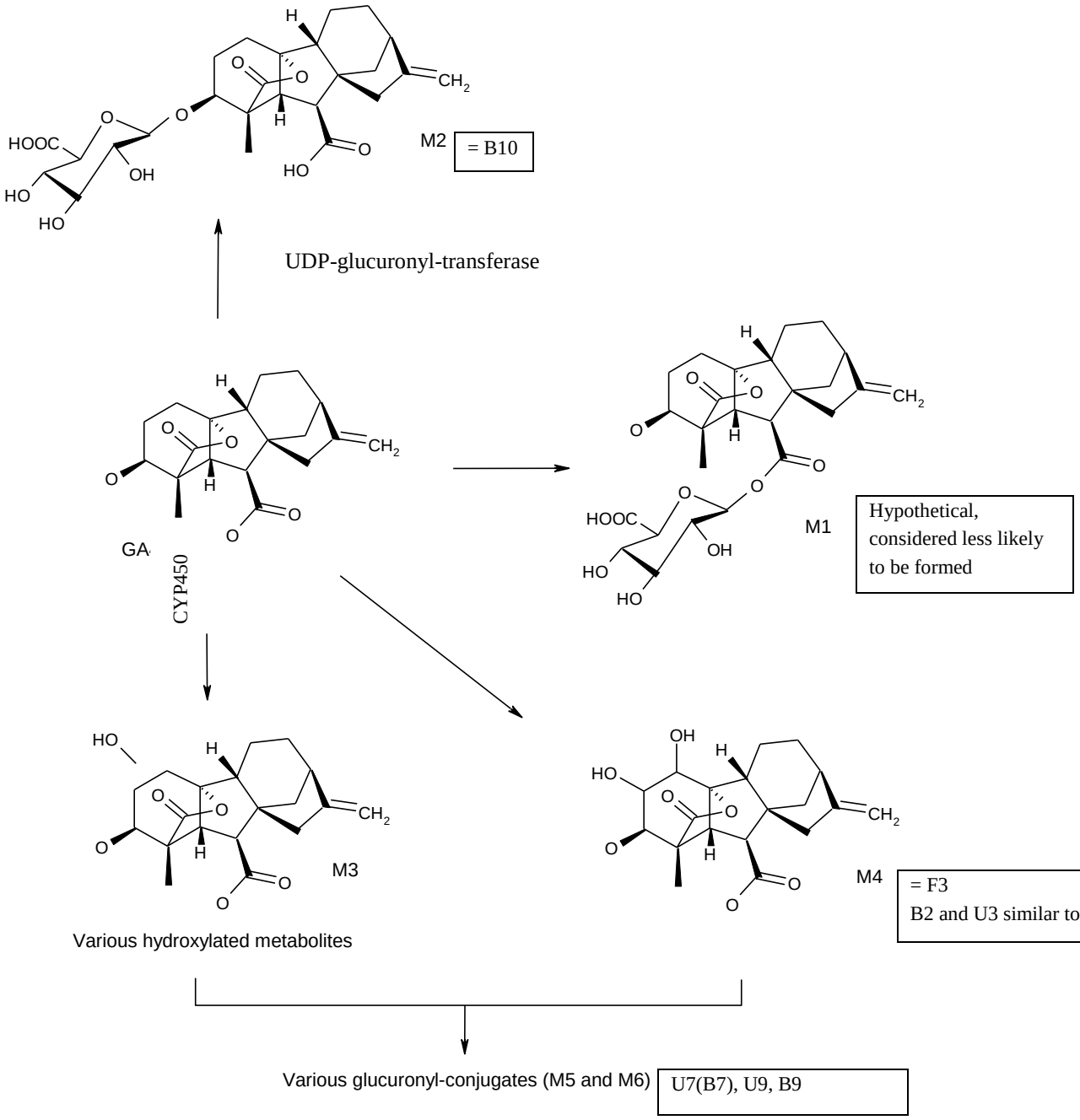
Table 6.1.1-5: Summary of identified metabolites in urine (U), feces (F) and bile (B) as % of dose administrated.

Sample Time	peak	U1	U2	U3	U4	U5	U6	U7	U8	U9	U10	U11	U12	U13	GA4	GA7	U14	Total
	RT	6.8	7.0	7.4	7.9	8.7	10.0	10.6	11.1	12.3	13.6	14.3	15.1	17.7	19.9	19.7	21.4	
	% dose																	
B1 female 0-8h	29.3	-		-	0.3	0.5	-	0.1	-	0.1	0.2	-	0.4	0.1	27.0	-	0.4	29.0
8-24h	9.9	-	-	-	0.1	0.2	-	-	-	-	0.1	-	0.2	-	9.0	-	0.1	9.6
Total 0-24h	39.2	-	-	-	0.3	0.7	-	0.1	-	0.1	0.3	-	0.5	0.2	36.0	-	0.5	38.7
B1 male 0-8h	5.6	-	-	0.1	0.4	0.4	-	0.6	0.2	0.8	-	-	0.3	0.1	2.5	-	-	5.3
8-24h	4.5	0.1	-	0.1	0.2	0.3	-	0.5	0.2	0.5	-	-	0.1	0.2	1.9	-	-	4.1
Total 0-24h	10.1	0.1	-	0.2	0.6	0.7	0.1	1.1	0.4	1.3	-	-	0.4	0.3	4.4	-	-	9.5
B2 male 0-8h	9.1	0.1	-	0.1	1.0	0.8	0.1	0.6	0.2	0.9	0.1	0.2	0.1	0.2	-	4.2	-	8.5
8-24h	6.3	0.1	-	0.3	0.8	0.6	-	0.5	0.1	0.4	0.1	0.2	0.1	0.2	-	2.4	-	5.8
Total 0-24h	15.4	0.2	-	0.4	1.8	1.5	0.1	1.1	0.3	1.3	0.2	0.4	0.2	0.4	-	6.6	-	14.4
C female 0-8h	31.9	-	-	-	0.2	0.3	-	0.1	-	0.1	0.2	-	0.2	0.1	30.2	-	0.3	31.6
8-24h	10.6	-	-	-	-	0.1	-	-	-	-	0.1	-	0.1	-	9.9	-	0.1	10.3
Total 0-24	42.6	-	-	-	0.2	0.5	-	0.1	-	0.1	0.2	-	0.4	0.1	40.0	-	0.4	42.1
C male 0-8h	8.3	-	-	-	0.3	0.3	-	0.6	0.2	0.9	0.1	-	0.4	0.1	4.8	-	-	7.8
8-24h	4.8	-	-	0.1	0.2	0.2	0.1	0.4	0.2	0.4	-	-	0.3	0.2	2.5	-	-	4.5
Total 0-24	13.1	-	-	0.1	0.5	0.6	0.1	1.0	0.4	1.4	0.1	-	0.7	0.2	7.4	-	-	12.4
D female 0-8h	52.4	-	-	-	0.3	0.4	-	0.1	-	0.2	0.2	-	0.3	-	50.4	-	0.1	52.0
8-24h	23.7	-	-	-	0.2	0.2	-	0.1	-	0.1	0.1	-	0.2	-	22.6	-	0.1	23.5
Total 0-	76.1	-	-	-	0.4	0.6	-	0.2	-	0.2	0.3	-	0.5	-	72.9	-	0.3	75.5
D male 0-8h	30.4	-	-	0.5	0.6	0.6	-	0.7	0.4	1.9	0.2	-	0.4	0.2	23.6	-	-	29.1
8-24h	12.0	-	0.2	0.3	0.3	0.3	0.1	0.4	0.2	0.9	0.1	-	0.3	0.1	8.8	-	-	11.8
Total 0-24h	42.4	-	0.2	0.8	0.9	0.8	0.1	1.1	0.5	2.8	0.2	-	0.7	0.3	32.5	-	-	40.8

- <0.1%

Sample pool	peak	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13	F14	GA4	GA7	Total
	RT	6.2	7	7.4	7.8	8.5	10	10.6	11.1	12.3	14.2	15.1	16.9	17.9	18.9	19.9	19.7	
	%dose																	
B1 female 0-24 h	53.4	-	-	1.9	-	2.7	-	1.1	-	-	-	3.4	0.5	-	0.9	40.3	-	50.8
24-48 h	6.1	0.1	-	0.1	-	0.2	-	-	-	-	-	-	0.1	-	-	5.4	-	5.9
Total 0-48 h	59.5	0.1	-	2.0	-	2.9	-	1.1	-	-	-	3.4	0.6	-	0.9	45.7	-	56.8
B1 male 0-24	88.6	4.2	0.7	13.1	1.3	2.6	3.4	4.7	2.2	2.8	-	1.2	0.8	0.5	1.0	46.4	-	84.9
24-48 h	8.3	0.3	0.1	1.1	-	0.2	0.2	0.4	0.1	0.1	-	-	-	-	0.1	5.7	-	8.2
Total 0-48 h	96.9	4.5	0.7	14.1	1.3	2.8	3.7	5.1	2.3	2.9	-	1.2	0.8	0.5	1.0	52.1	-	93.1
B2 male 0-24 h	77.4	1.5	1.2	4.8	2.3	4.6	1.2	3.1	0.7	0.9	3.8	-	0.5	4.1	-	-	42.7	71.3
24-48 h	4.7	0.1	0.1	0.3	-	0.2	-	0.1	-	-	0.4	-	-	0.4	-	-	2.7	4.4
Total 0-48 h	82.1	1.6	1.3	5.1	2.3	4.8	1.2	3.2	0.7	0.9	4.3	-	0.5	4.5	-	-	45.4	75.8
C female 0-24	46.3	-	-	1.0	0.3	1.9	-	0.8	-	-	-	0.3	0.3	-	0.7	38.0	-	43.3
24-48 h	13.4	0.1	-	0.4	0.1	0.4	-	0.1	-	-	-	-	0.1	-	-	12.0	-	13.1
Total 0-48 h	59.8	0.1	-	1.4	0.4	2.3	-	0.9	-	-	-	0.3	0.4	-	0.8	50.0	-	56.5
C male 0-24 h	67.1	3.4	1.3	12.3	1.2	2.0	2.9	3.9	1.9	-	-	-	-	0.4	0.8	30.5	-	60.5
24-48 h	22.5	1.4	0.2	3.9	0.4	0.5	1.2	1.1	0.6	-	-	-	-	0.1	0.1	12.3	-	21.8
Total 0-48 h	89.6	4.8	1.5	16.3	1.7	2.5	4.1	5.0	2.5	-	-	-	-	0.4	0.9	42.7	-	82.3
D female 0-24 h	22.8	0.3	0.2	0.8	0.2	0.9	-	0.7	-	0.1	-	10.2	-	-	0.9	7.1	-	21.5
24-48 h	4.8	-	-	0.1	-	0.2	-	0.1	-	-	-	1.1	-	-	0.1	2.9	-	4.5
Total 0-48 h	27.6	0.3	0.2	1	0.2	1.2	-	0.8	-	0.1	-	11.3	-	-	1.1	9.9	-	26.1
D male 0-24 h	57.1	1.5	5.9	4.9	0.9	1.8	1.1	3.0	0.7	0.9	0.3	14.6	1.9	-	1.0	13.9	-	52.3
24-48 h	3.6	-	-	0.5	-	0.1	0.1	0.1	-	-	-	0.4	-	-	-	2.3	-	3.4
Total 0-48 h	60.8	1.5	5.9	5.4	0.9	1.9	1.2	3.1	0.7	0.9	0.3	14.9	1.9	-	1	16.2	-	55.7
Bile		**B1**	**B2**	**B3**	**B4**	**B5**	**B6**	**B7**	**B8**	**B9**	**B10**	**B11**	**B12**	**B13**	**B14**	**GA4**	**GA7**	**Total**
G male 0-12 h	70.2	-	1.8	2.8	1.4	1.5	-	6.8	-	2.2	10.5	36.3	-	-	-	3.6	-	66.9
G female 0-12 h	53.8	0.1	0.2	0.1	0.1	0.8	-	0.5	-	0.5	9.7	34.0	-	-	-	5.2	-	51.2

- : < 0.1%



b) <u>Evaluation of additional data for the purpose of renewal of Annex I inclusion</u>

No additional data was submitted or requested for the purpose of renewal of approval of GA4/7.

B.6.1.2. Absorption, distribution, metabolism and excretion by other routes

Regulation (EU) No 283/2013 specifies that comparative *in vitro* metabolism studies should be performed on animal species to be used in pivotal studies and on human material in order to determine the relevance of the toxicological data from animal studies for the human risk assessment. The main metabolic pathways identified in the rat study (i.e. hydroxylation and conjugation with glucuronic acid) are common to humans, and gibberellins GA4/7 are widely found in plants and fruit, are a normal component of a healthy diet, and very low toxicity was observed in the available toxicity studies. On the basis that the metabolic pathway is common to both rats and humans and is a simple, common pathway; variations in the metabolism of gibberellins GA4/7 are not expected. A review paper is summarised in this dossier that demonstrates the widespread natural occurrence of gibberellins in plants, fungi and bacteria (MacMillan, 2002; CA 8.2.2/01): 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. Based on the ubiquitous nature of gibberellins GA4/7, further *in vitro* investigation of the metabolism is not considered necessary. As the main metabolic pathways identified in the rat are common to humans, the formation of novel or toxic metabolites is not expected and therefore *in vitro* investigation of metabolism (e.g. using cultured human liver cells) is unlikely to provide additional information relevant to the risk assessment. In the available ADME study ([redacted] *et al*, 2000), collected rat urine and bile samples were incubated with ß-glucuronidase and arylsulphatase/ ß-glucuronidase (enzymes present in humans) in order to detect glucuronic acid or sulphate conjugated metabolites. Sulphate metabolites were not detected. Glucuronyl metabolites were detected in urine and bile confirming that metabolism is predominantly restricted to non-toxic mono- and di-hydroxyls, and glucuronide conjugates of parent and hydroxyls; novel metabolites were not detected. Hydroxylation is a fairly simple metabolic process that occurs in both rats and humans and is driven largely by cytochrome P-450; there is no evidence to suggest that this metabolic pathway in humans would differ significantly from that observed in the rat. On this basis, further data are not considered necessary.

RMS comments and conclusion:

Comparative in vitro metabolism studies listed in EU data requirements for active substances (Commission Regulation No 283/2013) were not provided.
The main metabolic pathways identified in the rat *in vivo* study are common to humans, thus the formation of novel or toxic metabolites is not expected. However, the differences between the two organisms could be in the amount of the produced metabolite. GA4/7 is present widely in plant and fruits, the exposure to GA4/7 for the PPP is expected to be low, 0-2 – 1.3% ADI see (Vol1, 2.7.9).

The RMS is of the opinion that the lack of ADME and comparative in vitro metabolism studies do not have major influence on the outcome of the toxicological risk assessment of GA4/7 because:
-metabolic transformation of GA4/7 leads to detoxification process, which facilitate urine and biliary excretion,
- the same detoxification metabolism can be predicted also for humans,
- GA4/7 is of low toxicity
- human exposure is predicted to be low (being less or around to background levels for consumers)
- the missing toxicological information (metabolism studies) is taken into account in the setting of reference doses

B.6.2. ACUTE TOXICITY

RMS comment: Acute oral toxicity studies in rats, acute dermal toxicity studies in rat and rabbits, acute inhalation toxicity studies in rats, skin and eye irritation studies in rabbits and skin sensitisation study in guinea pigs were performed with GA4/7 and submitted for the first review of GA4/7. In addition, a phototoxicity study was submitted for the renewal of approval. An overall RMS comment can be found at the end of each set of acute toxicity studies.

B.6.2.1. Oral

a) Previous evaluation (2005-2011)

Acute oral toxicity data were submitted during the EU review of GA4/7 and are available in the EU DAR. The studies below were considered acceptable in the EFSA conclusion and are considered adequate for supporting renewal of GA4/7; no new acute toxicity studies are submitted. EFSA (2012) concluded that the acute oral LD_{50} of GA4/7 is >5000 mg/kg bw in the rat.

B.6.2.1/01

PREVIOUS EVALUATION	This study was evaluated in the original DAR and has been considered by EFSA.
Data point addressed:	CA 5.2.1/01 (B.6.2.1.1 DAR)
Author(s) (year):	[redacted] (1997)
Title:	GA4/7 99% technical: Acute oral toxicity to the rat
Laboratory report / project number:	[redacted]
Testing facility:	[redacted]
Published:	No
Test guideline used:	OECD 401, EEC Method B.1, US EPA 152-10
Deviations:	The study was conducted to a now deleted guideline, at a limit dose higher than is currently recommended. However it is acceptable for hazard classification.
GLP:	Yes
EU Agreed Endpoint:	Rat LD_{50} oral > 5000 mg/kg bw

Executive summary

In a preliminary acute toxicity study, a group of 2 male and 2 female Sprague-Dawley rats were given a single oral dose (gavage) of 3200 mg/kg bw gibberellins GA4/7 technical (purity 99%; GA4 >90%, batch D105). There were no deaths, and clinical signs comprised piloerection, hunched posture, partially closed eyelids and unsteadiness in all rats. These had all resolved by Day 3. The main study was then carried out with 5 rats/sex at a dose level of 5000 mg/kg bw. There were no deaths during the study. Piloerection was observed in all rats within two minutes of dosing. This sign persisted and was accompanied by hunched posture (all rats) and ungroomed appearance (females). Recovery, as judged by external appearance and behaviour was complete by either Day 2 (males) or Day 5 (females). A slight low bodyweight gain was evident for one female on Day 8 only. All other

rats were considered to have achieved satisfactory bodyweight gains during the study. No macroscopic abnormalities were observed for animals killed on Day 15.

I Materials and Methods

A. Materials:

1.	**Test Material**	Gibberellins GA4/7 (GA4/7 Technical)
	Description	White powder
	Lot/Batch	D105
	Purity	GA4 >90%, <10% w/w GA7 and other gibberellins, (99% GA4/7)
	Stability	Assumed to be stable for the duration of the study
2.	**Vehicle**	Distilled water
3.	**Test animals**	Rats
	Species	Sprague-Dawley origin (Hsd/Ola:Sprague-Dawley (CD))
	Age	Approximately 8 to 12 weeks old
	Weight	204-228 g
	Source	[redacted]
	Acclimation period	5 days
	Diet	Standard Lab rodent diet (Biosure LAD 1), *ad libitum*
	Water	Tap water *ad libitum*
	Housing	Animals were housed in groups of up to five rats of the same sex in metal cages with wire mesh floors

4. Environmental conditions

Temperature	22 ± 3°C
Humidity	30-70%
Air changes	10-15 air changes/hour
Photoperiod	12 hour light/dark cycle

B. Study Design and Methods

1. In life dates: 23 July to 13 August 1996

2. Animal assignment and treatment

A preliminary study was conducted by dosing two male and two female rats at 3200 mg/kg bw. For the main study, a group of ten rats (five/sex) and administered a single oral dose of 5000 mg/kg bw gibberellins GA4/7 by oral gavage. The test substance was administered in distilled water at a volume of 10 mL/kg bw, and rats were fasted overnight prior to administration. Animals were observed soon after dosing at frequent intervals for the remainder of Day 1 (a period of 3 hours). Animals were observed twice daily for the remainder of the experiment. Body weights for each rat were recorded on Days 1 (prior to dosing), 8 and 15. On Day 15, surviving animals were sacrificed and all animals were necropsied and examined for gross pathological changes.

3. Statistics The data do not warrant statistical analysis

II Results and Discussion

A. Mortality No mortalities occurred. The oral LD_{50} for males was > 5000 mg/kg bw

		for females was > 5000 mg/kg bw combined was > 5000 mg/kg bw
B.	Clinical Observations	Piloerection was observed in all rats within two minutes of dosing. This sign persisted and was accompanied by hunched posture (all rats) and ungroomed appearance (females). Recovery, as judged by external appearance and behaviour was complete by either Day 2 (males) or Day 5 (females).
C.	Body weight	A slight low bodyweight gain was evident for one female on Day 8 only. All other rats were considered to have achieved satisfactory bodyweight gains during the study.
D.	Necropsy	No macroscopic abnormalities were observed
E	Deviations	Animals in the main study were fed approximately three hours after dosing instead4 hours as cited in the study protocol. The deviation was a result of unavoidable delay in formulation preparation and was not considerd to have affected the validity and integrity of the study.

Conclusion

The acute oral LD_{50} was found to be greater than 5000 mg/kg bw in male and female rats. Classification according to Regulation (EC) No 1272/2008 is not required. The endpoint was agreed in the EFSA Conclusion and is still considered valid.

B.6.2.1/02

PREVIOUS EVALUATION	This study was evaluated in the DAR and has been considered by EFSA.
Data point addressed:	CA 5.2.1/02 (B.6.2.1.1 DAR)
Author(s) (year):	[redacted] (1988)
Title:	Acute oral toxicity study of Gibberellins A4A7 (GA_4A_7) in rats
Laboratory report / project number:	[redacted]
Testing facility:	[redacted]
Published:	No
Test guideline used:	US EPA FIFRA Guideline 81-1; equivalent to OECD 401 (now deleted)
Deviations:	The study employed a limit dose higher than is currently recommended, however it is acceptable for hazard classification
GLP:	Yes
EU Agreed Endpoint:	Rat LD_{50} oral > 5000 mg/kg bw

Executive summary

A group of fasted Sprague-Dawley rats (5 male and 5 female) were given a single oral dose (gavage) of 5000 mg/kg bw gibberellins GA4/7 (batch no 16-213-CD) in distilled water. There were no deaths during the study.

Clinical signs of toxicity were limited to diarrhoea in three females up to 4 hours after dosing. No abnormalities were recorded at the macroscopic examination on Day 14.

I Materials and Methods

A. Materials:

1.	**Test Material**	Gibberellins GA4/7
	Description	White powder
	Lot/Batch	16-213-CD
	Purity	Not stated in report but quoted as 90.0% for same batch in other reports
	Stability	Assumed to be stable for the duration of the study
2.	**Vehicle**	Distilled water
3.	**Test animals**	Rats
	Species	Sprague-Dawley Albino Crl:CD(SD)BR
	Age	Young adult
	Weight	204 - 210 g males; 216-248 g females
	Source	[redacted]
	Acclimation period	7 days
	Diet	Standard Lab rodent diet (Purina Rodent Chow #5001), *ad libitum*
	Water	Tap water *ad libitum*
	Housing	Animals were housed in same sex groups of 5
4.	**Environmental conditions**	
	Temperature	~19-25°C
	Humidity	49-66%
	Air changes	Not stated
	Photoperiod	Alternating 12 hour light and dark cycle

B. Study Design and Methods

1. In life dates: 7 to 21 July 1988

2. Animal assignment and treatment

A group of ten rats (five/sex) were fasted overnight and administered a single oral dose of 5000 mg/kg bw gibberellins GA4/7 by oral gavage. The test substance was administered in distilled water at a volume of 10 mL/kg bw. Animals were observed for clinical signs and mortality at 1, 2.5 and 4 hours after dosing; animals were observed daily thereafter for clinical signs and twice daily for mortality, for 14 days. Body weights were recorded the day of dosing, on days 1 and at study termination. All rats were sacrificed at the end of the 14 day observation period and subjected to a gross necropsy.

3. Statistics The data do not warrant statistical analysis

II Results and Discussion

A. Mortality No mortalities occurred. The oral LD_{50}
for males was > 5000 mg/kg bw
for females was > 5000 mg/kg bw
combined was > 5000 mg/kg bw

B.	Clinical Observations	Three females developed diarrhoea approximately 2 hours after dosing and this persisted at the 4 hour post dosing observation. Recovery, as judged by external appearance and behaviour, was complete by Day 1.
C.	Body weight	All animals gained weight during the observation period.
D.	Necropsy	No macroscopic abnormalities were observed

Conclusion

The acute oral LD_{50} of gibberellins GA4/7 was found to be greater than 5000 mg/kg bw in male and female rats. Classification of gibberellins GA4/7 according to Regulation (EC) No 1272/2008 is not required. The endpoint was agreed in the EFSA Conclusion and is still considered valid.

RMS comments and conclusion:

The two studies on acute oral toxicity were submitted during the previous EU review of GA4/7. The studies followed the OECD TG 401 (1987) and GLP principles. OECD TG 401 was deleted in December 2002. Since then, OECD TG 420/423/425 for the evaluation of the acute toxicity potential of test items are used in order to minimize the number of animals. The purity of the a.s.in the first study was defined as >90% w/w (GA4). No information on the a.s. purity was found in the second study, however from the oral prenatal developmental study ([redacted] 1989) and acute inhalation study ([redacted] J.B. (1988)) done using the same batch it can be assumed, that the purity was 90% w/w GA4/7. The LD_{50} endpoint derived by the older guideline is still adequate and can be used for classification purposes according to CLP Regulation (EC) No 1272/2008. The acute oral LD_{50} value for fasted rats (males, females, combined) was > 5000 mg/kg bw in all the presented studies.

No classification regarding acute oral toxicity is required for GA4/7 according to criteria laid down in Regulation (EC) No 1272/2008; the test substance is not subject to labelling requirements.

c) <u>Evaluation of additional data for the purpose of renewal of Annex I inclusion</u>

No additional data was submitted or requested for the purpose of renewal of approval of GA4/7.

B.6.2.2. Dermal

a) Previous evaluation (2005-2011)

Acute dermal toxicity data were submitted during the EU review of GA4/7 and are available in the EU DAR. The studies were below considered acceptable in the EFSA conclusion and are considered adequate for supporting renewal of GA4/7; no new acute toxicity studies are submitted. EFSA (2012) concluded that the acute dermal LD_{50} of GA4/7 is >2000 mg/kg bw in the rat.

B.6.2.2/01

PREVIOUS EVALUATION	This study was evaluated in the DAR and has been considered by EFSA.
Data point addressed:	CA 5.2.2/01 (B.6.2.1.2 DAR)
Author(s) (year):	[redacted] (1997)

Title:	GA4/7 99% technical: Acute dermal toxicity to the rat
Laboratory report / project number:	[redacted]
Testing facility:	[redacted]
Published:	No
Test guideline used:	OECD 402, EEC Method B.3, US EPA 152-11
Deviations:	None
GLP:	Yes
EU Agreed Endpoint:	Rat LD_{50} dermal > 2000 mg/kg bw

Executive summary

A group of CD rats of Sprague Dawley origin (5 male and 5 female) were given a single dermal dose of gibberellins GA4/7 technical (purity 99%; GA4 >90%, batch D105). The test substance (moistened with distilled water) was applied to each animal's back and held in place for 24 hours with an occlusive dressing. There were no deaths and no signs of systemic reaction to treatment. Dermal irritation consisted of slight erythema in one female rat on Day 2 only. Notably low bodyweight gains evident on Day 8 for two females. All other rats were considered to have achieved satisfactory bodyweight gains during the study. No visible lesions were observed at gross necropsy on Day 14.

I Materials and Methods

A. Materials:

1. Test Material Gibberellins GA4/7 (GA4/7 Technical)

Description: White powder

Lot/Batch: D105

Purity: GA4 >90%, <10% w/w GA7 and other gibberellins, (99% GA4/7)

Stability: Assumed to be stable for the duration of the study

2. Vehicle Distilled water

3. Test animals Rats

Species: Sprague-Dawley origin (Hsd/Ola:Sprague-Dawley (CD))

Age: Approximately 7 to 10 weeks old

Weight: 223-248 g

Source: [redacted]

Acclimation period: 18 days

Diet: Standard Lab rodent diet (SDS LAD 1), *ad libitum*

Water: Tap water *ad libitum*

Housing: Animals were housed individually in metal cages with wire mesh floors

4. Environmental conditions

Temperature: 22 ± 3°C

Humidity: 30-70%

Air changes: 10-15 air changes/hour

Photoperiod 12 hour light/dark cycle

B. Study Design and Methods

1. In life dates: 29 July to 12 August 1996

2. Animal assignment and treatment

Animals were randomly allocated to the test groups. On the day prior to dosing hair was removed from the dorso-lumbar region of each rat with electric clippers exposing an area equivalent to approximately 10% of the total body surface. The test substance was prepared at a maximum practical concentration of 62.5% w/v in distilled water and administered at a volume of 3.2 mL/kg bw; the test substance was prepared on the day of dosing. The test substance was administered as a single occluded dermal application and was promptly covered with gauze that was held in place with a non-irritant occlusive dressing encircling firmly around the trunk. After an exposure period of 24 hours, the occlusion was removed and residual test material was washed with warm (30° to 40°C) water and blotted dry with absorbent paper. Day of dosing was designated Day 1. No control animals were included in this study. Mortality checks were conducted twice daily. Animals were observed soon after dosing and at frequent intervals for the remainder of day 1 (about 5 hours) for clinical signs. On subsequent days animals were observed twice daily for clinical signs. Animals were observed for 14 days after dosing.

3. Statistics The data do not warrant statistical analysis

II Results and Discussion

A.	Mortality	No mortalities occurred. The dermal LD_{50} for males was > 2000 mg/kg bw for females was > 2000 mg/kg bw combined was > 2000 mg/kg bw
B.	Clinical Observations	There were no clinical signs of systemic reaction to treatment. Dermal irritation consisted of slight erythema in one female rat on Day 2 only.
C.	Body weight	Notably low bodyweight gains evident on Day 8 for two females. All other rats were considered to have achieved satisfactory bodyweight gains during the study.
D.	Necropsy	No treatment-related gross necropsy observations were noted.

Conclusion

The acute dermal LD_{50} was found to be greater than 2000 mg/kg bw in male and female rats. Classification of gibberellins GA4/7 according to Regulation (EC) No 1272/2008 is not required. The endpoint was agreed in the EFSA Conclusion and is still considered valid.

B.6.2.2/02

PREVIOUS EVALUATION	This study was evaluated in the DAR and has been considered by EFSA.
Data point addressed:	CA 5.2.2/02 (B.6.2.1.2 DAR)
Author(s) (year):	[redacted] (1988)
Title:	Acute dermal toxicity study of Gibberellins A4A7 (GA_4A_7) in rabbits
Laboratory report / project number:	[redacted]
Testing facility:	[redacted]

Published:	No
Test guideline used:	US EPA FIFRA Guideline 81-2, equivalent to OECD 402
Deviations:	None
GLP:	Yes
EU Agreed Endpoint:	Rat LD_{50} dermal > 2000 mg/kg bw

Executive summary

A group of New Zealand white rabbits (5 male and 5 female) were given a single dermal dose of 2000 mg/kg bw gibberellins GA4/7 (batch no 16-213-CD). The test substance (moistened with 0.9% saline) was applied to clipped skin and covered by a gauze patch held in place for 24 hours with an occlusive dressing. There were no deaths and no clinical signs of toxicity. Dermal reactions were transient (fully recovered by Day 7) and did not exceed a well-defined response.

Materials and Methods

A. Materials:

1. Test Material Gibberellins GA4/7

Description: White powder

Lot/Batch: 16-213-CD

Purity: Not stated in report but quoted as 90.0% for same batch in other reports

Stability: Assumed to be stable for the duration of the study

2. Vehicle 0.9% saline

3. Test animals Rabbits

Species: New Zealand White rabbits (Hra: (NZW)SPF)

Age: Young adult

Weight: 2346-2692 g males; 2474-2640 g females

Source: [redacted]

Acclimation period: 7 days

Diet: Standard Lab diet for rabbits (Purina High Fiber Rabbit Chow #5326), measured amount

Water: Tap water *ad libitum*

Housing: Individually in screen-bottom cages

4. Environmental conditions

Temperature: 19-24°C

Humidity: 58-73%

Air changes: Not stated

Photoperiod: Not stated

B. Study Design and Methods

1. In life dates: 7 to 21 July 1988

2. Animal assignment and treatment

A group of rabbits (5 male and 5 female) were given a single dermal dose of 2000 mg/kg bw gibberellins GA4/7. Approximately 24 hours before testing, the hair on the dorso-lumbar region of each animal was removed using clippers. The test substance was moistened with 0.9% saline and applied to the clipped dorsal skin and held in place with an occlusive dressing. The dressing was removed after 24 hours and the test site washed with warm water and paper towels. Animals were observed for 14 days for mortality and clinical signs. Body weights were recorded immediately before application of the test substance, on day 7 and at the end of the 14 day observation period. Rabbits were sacrificed at the end of the observation period and subjected to a gross necropsy.

3.	**Statistics**	The data do not warrant statistical analysis

II Results and Discussion

A.	Mortality	No mortalities occurred. The dermal LD_{50} for males was > 2000 mg/kg bw for females was > 2000 mg/kg bw combined was > 2000 mg/kg bw
B.	Clinical Observations	No clinical signs of reaction to treatment were observed. The majority of rabbits developed slight or well-defined erythema at the dose site on Day 1. Reactions had largely resolved by Day 3, slight reactions only remaining for two rabbits. No other dermal changes were apparent.
C.	Body weight	All animals gained weight during the observation period.
D.	Necropsy	No treatment-related gross necropsy observations were noted.

Conclusion

The acute dermal LD_{50} was found to be greater than 2000 mg/kg bw in male and female rabbits. Classification of gibberellins GA4/7 according to Regulation (EC) No 1272/2008 is not required. The endpoint was agreed in the EFSA Conclusion and is still considered valid.

RMS comments and conclusion:

The two studies on acute dermal toxicity (one on rat and one on rabbits) were submitted during the previous EU review of GA4/7. The studies are acceptable. The studies followed the OECD 402 (1987) or guideline equivalent to OECD 402 and GLP principles. A new version of OECD 402 was adopted in November 2017 in order to minimize the number of animals needed for the conduction of acute derma toxicity study if the waving of the study is not possible. According to Commission Regulation (EU) No 283/2013 (Annex, Point 5.2.2.) the acute dermal toxicity study could be waived due to oral LD_{50} being more than 2000 mg/kg bw, thus leading to no classification according to CLP Regulation (EC) No 1272/2008. However, the LD_{50} endpoint derived by the older guideline is still adequate and can be used for classification purposes according to CLP Regulation (EC) No 1272/2008. The acute dermal LD_{50} value for rats and rabbits (males, females, combined) was > 2000 mg/kg bw in all the presented studies.

No classification regarding acute dermal toxicity is required for GA4/7 according to criteria laid down in Regulation (EC) No 1272/2008; the test substance is not subject to labelling requirements.

b) <u>Evaluation of additional data for the purpose of renewal of Annex I inclusion</u>

No additional data was submitted or requested for the purpose of renewal of approval of GA4/7.

B.6.2.3. Inhalation

a) Previous evaluation (2005-2011)

Acute inhalation toxicity data were submitted during the EU review of GA4/7 and are available in the EU DAR. The studies below were considered acceptable in the EFSA conclusion and are considered adequate for supporting renewal of GA4/7; no new acute toxicity studies are submitted. EFSA (2012) concluded that the acute 4-hour inhalation LC_{50} of GA4/7 is >2.98 mg/L in the rat.

B.6.2.3/01

PREVIOUS EVALUATION	This study was evaluated in the DAR and has been considered by EFSA.
Data point addressed:	CA 5.2.3/01
Author(s) (year):	[redacted] (1997) (B.6.2.1.3 DAR)
Title:	Acute inhalation toxicity to rats of GA4/7 99% technical
Laboratory report / project number:	[redacted]
Testing facility:	[redacted]
Published:	No
Test guideline used:	OECD 403, US EPA, EEC and Japan MAFF guidelines for acute inhalation
Deviations:	None
GLP:	Yes
EU Agreed Endpoint:	Rat LC_{50} > 5.44 mg/L

Executive summary

In an acute inhalation toxicity study, a group of Sprague Dawley rats (5/sex) were exposed (4-hour, nose only exposure) by the inhalation route to a dust atmosphere of gibberellins GA4/7 technical (purity 99%; GA4 >90%, batch D105) at a concentration of 5.44 mg/L. The MMAD was 4.4 µm with a geometric standard deviation of 3.36. There were no deaths during the study. Signs related to treatment included brown staining on the head, exaggerated respiratory movements, matted fur, soiled fur and test substance on the fur. Recovery was complete by Day 3. Bodyweight gain was slightly reduced for Day 1 only; bodyweight gain was otherwise normal for the remainder of the study. There were no observable abnormalities at gross necropsy.

I Materials and Methods

A. Materials:

1. Test Material Gibberellins GA4/7

Description White solid

Lot/Batch D105

Purity GA4 >90%, <10% w/w GA7 and other gibberellins, (99% GA4/7)

Stability Assumed to be stable for the duration of the study

2. Vehicle None

3. Test animals Rats

Species	Sprague-Dawley
Age	Approximately 8 weeks old
Weight	246-268 g (males); 208-227 g (females)
Source	[redacted]
Acclimation period	5 days
Diet	Standard Lab rodent diet ([redacted]), *ad libitum*
Water	Tap water *ad libitum*
Housing	Animals were housed in groups of up to five rats of the same sex in suspended metal cages with wire mesh floors

4. Environmental conditions

Temperature	17-25°C
Humidity	42-76%
Air changes	Not stated
Photoperiod	12 hour light/dark cycle

B. Study Design and Methods

1. In life dates: 10 to 31 July 1996

2. Animal assignment and treatment

Rats (5/sex) were exposed nose only and were observed continuously for signs of toxicity during the 4-hour exposure period. Clinical signs were recorded at the end of the chamber equilibration period, at 0.25, 0.5 and 1.0 hours and then at hourly intervals during the exposure. During the observation period, the clinical signs were recorded once/day. Body weights were recorded daily from the day of delivery until the end of the observation period. Food and water consumption was measured daily from the day following arrival. At the end of the 14-day observation period rats were sacrificed and subjected to a detailed macroscopic examination. The lungs were removed, dissected clear and weighed. Lungs were infused with, and preserved in buffered 10% formalin together with samples of the liver and kidneys for possible microscopic examination.

3. Generation of test atmosphere/chamber description

A 30 L nose only exposure chamber was used. A Wright dust generator was used to produce the test atmospheres. The test material was ball-milled for approximately 24 hours and passed through a 63 µm sieve. The dust generator containing the milled test material was positioned on a stand at the side of the exposure chamber. The air flow rate was 25 litres/minute.

Five air samples were taken from the chamber during the exposure to determine the concentration of gibberellins GA4/7 in the chamber air by gravimetric analysis. Samples were collected at 30, 60, 120, 180 and 230 minutes following the equilibration period. Two additional air samples were taken during the exposure using a Marple cascade impactor. The material collected on the stages of the sampler was weighed to determine the particle size distribution of the test substance in the test atmosphere. The samples were collected at 90 and 210 minutes following equilibration.

Mass median aerodynamic diameter: 4.4 µm, geometric standard deviation: 3.36, percent respirable particles (<7 µm) = 65%. The MMAD was marginally larger than the ideal upper size (4 µm) for an acute inhalation study. The estimated exposure concentration for an MMAD of 4.0 µm was 5.12 mg/L.

4. Statistics The data do not warrant statistical analysis

II Results and Discussion

A.	Mortality	No mortalities occurred. The 4-hour inhalation LC_{50} for males was > 5.44 mg/L for females was > 5.44 mg/L combined was > 5.44 mg/L
B.	Clinical Observations	During exposure: No clinical signs indicative of a toxic or irritant effect were noted. Soiling of the fur with excreta, as a consequence of the method of restraint, was noted. During observation period: Signs related to treatment included brown staining on the head, exaggerated respiratory movements, matted fur, soiled fur and test substance on the fur. Recovery was complete by Day 3.
C.	Body weight	All rats gained weight during the 14 day observation period. The rate of body weight gain was reduced for 1 day only following exposure.
D.	Food and water consumption	There was a reduction in food consumption on the day following exposure; the reduction was attributed to the exposure procedure. Food consumption was otherwise normal during the observation period. Water consumption was unaffected by treatment.
E.	Necropsy	No macroscopic abnormalities were observed. The lung weight to body weight ratios were within normal limits.

Conclusion

The acute 4-hour inhalation LC_{50} was found to be greater than 5.44 mg/L in male and female rats. Classification according to Regulation (EC) No 1272/2008 is not required. The endpoint was agreed in the DAR and is still considered valid.

B.6.2.3/02

PREVIOUS EVALUATION	This study was evaluated in the DAR and has been considered by EFSA.
Data point addressed:	CA 5.2.3/02 (B.6.2.1.3 DAR)
Author(s) (year):	[redacted] (1988)
Title:	Acute inhalation toxicity study with gibberellins A4A7 (GA4A7) in the rat
Laboratory report / project number:	[redacted]
Testing facility:	[redacted]
Published:	No
Test guideline used:	US EPA FIFRA Guideline 81-3, comparable to OECD 403
Deviations:	The test concentration is lower than that required by EU guidelines, however it is reported to be the maximum attainable concentration and is therefore acceptable for hazard classification

GLP:	Yes
EU Agreed Endpoint:	Rat LC_{50} > 2.98 mg/L

Executive summary

In an acute inhalation toxicity study, a group of Sprague Dawley rats (5/sex) were exposed (4-hour, whole-body exposure) by the inhalation route to a dust atmosphere of gibberellins GA4/7 (purity 90%, batch no 16-213-CD) at a concentration of 2.98 mg/L, the maximum attainable dose level for this study design. The MMAD was 5.83 µm with a geometric standard deviation of 1.67. There were no deaths during the study. Signs related to treatment observed on the day of exposure included compound on fur, urine stains, squinted eyes, crust on the eyes and nose, lacrimation, salivation and rhinorrhoea. Alopecia and crust on the eyes and nose were noted during the two-week observation period, however all animals were normal by Day 15. All animals gained bodyweight over the 14-day observation period. There were no observable abnormalities at gross necropsy.

I Materials and Methods

A. Materials:

1. Test Material Gibberellins GA4/7

Description: White powder

Lot/Batch: 16-213-CD

Purity: 90%

Stability: Assumed to be stable for the duration of the study

2. Vehicle

3. Test animals Rats

Species: Sprague-Dawley Crl:CD®BR

Age: 58 days males and 73 days females

Weight: 277.4-306.5 g males; 224.1-241.9 g females

Source: [redacted]

Acclimation period: 14 days

Diet: Standard Lab rodent diet (Purina Certified Rodent Laboratory Chow #5002), *ad libitum* except during exposure

Water: Tap water *ad libitum*

Housing: Animals were housed individually of in groups of up to 5 in suspended stainless steel cages with mesh floors.

4. Environmental conditions

Temperature: 20-22°C

Humidity: 45-60%

Air changes: Not stated

Photoperiod: 12 hour light/dark cycle

B. Study Design and Methods

1. In life dates: 25 July to 8 August 1988

2. Animal assignment and treatment

Five male and five female healthy rats were assigned to a single test group exposed to a nominal target dose of 5

mg/L. The animals were given a detailed physical examination prior to exposure and were checked for clinical signs of reaction to treatment at 30 and 60 minutes after exposure and then once daily for the remainder of the 14-day observation period. The animals were checked at hourly intervals during exposure, although the dust build-up did obscure the animals on some occasions. Mortality and moribundity checks were completed twice daily throughout the study. Body weights were recorded prior to dosing on Day 1 and on Day 8 and at termination on Day 15. Rats were subject to macroscopic necropsy and examination of external surfaces, cranial, thoracic and abdominal cavities with their organs and tissues, for gross pathological changes.

3. Generation of test atmosphere/chamber description

A 100 L plexiglass exposure chamber was operated in a dynamic mode with an airflow of 56 L/min. The design provided a whole body exposure. The atmosphere was generated by use of a Spengler particle generator. Powdered test material was packed into 4 individual 1 L Spengler cups using a 5000 psi press for 30 seconds. Air with a back pressure of 30 psi was directed through a flow meter to the dust generator at 56 L/min and the resultant undiluted dust-laden atmosphere was directed into the exposure chamber. Following 4 hours exposure to the test atmosphere, clean air was directed through the chamber for 30 minutes after which the animals were removed. The test atmosphere concentration in the exposure chamber was determined gravimetrically by collection of dust on pre-weighed Gelman glass-fibre filters using a Thomas Industries Sprayit pump operated at 3.8 L/min for 4 minutes. Exposure level concentration was calculated by taking pre-collection filter weight from the post-collection weight and dividing by the sample volume. An additional gravimetric sample was collected to determine the spatial homogeneity of the generated test atmosphere. Particle size distribution was determined twice during exposure by standard sampling techniques using an Andersen Cascade Impactor. Mass median aerodynamic diameter (MMAD) and the geometric standard deviation (GSD) were estimated from standard second order polynomial least squares fit of the logarithm for each stage constant versus cumulative weight distribution of the experimental data. Nominal exposure concentration was derived from the total quantity of test material used (determined by pre and post exposure weighing of various components of the atmosphere generation system) divided by airflow through the chamber. The chamber conditions (temperature and relative humidity) were monitored continuously through the exposure period and airflow rates were checked at 30 minute intervals.

Mass median aerodynamic diameter: 5.83 µm, geometric standard deviation: 1.67.

The MMAD value for the maximum attainable exposure level was greater than 4 µm. No data are presented for particle size distribution despite indications in the report that the information was recorded. These deviations did not compromise the integrity of the calculated LC50 value or the acceptability of the study

4. Statistics Analysis of particle size data was by a computerised particle size distribution analysis program. Median lethal dose calculations were subject to probit analysis where appropriate

II Results and Discussion

A. Mortality

No mortalities occurred. The 4-hour inhalation LC_{50}

for males was > 2.98 mg/L

for females was > 2.98 mg/L

combined was > 2.98 mg/L

B. Clinical Observations

Clinical signs for animals that were visible during exposure were limited to the presence of test material on the fur. After removal from the inhalation chamber all rats had compound on the fur and three cases of crusts on the eyes/nose and less common occurrence of rhinorrhoea, salivation and urine stains at 30 and 60 minutes after dosing. Lachrymation and squinting eyes were also noted in one or two rats 60 minutes after dosing. Crusts on eyes/nose persisted as the only sign from day 2 to 5 and from day 6 to 14 there were one or two rats showing alopecia

		(eyelids/paw/leg). All animals were overtly normal by Day 15.
C.	Body weight	All animals gained weight during the observation period.
D.	Necropsy	No macroscopic abnormalities were detected at necropsy.

Conclusion

The acute 4-hour inhalation LC_{50} was found to be greater than 2.98 mg/L in male and female rats. Classification of gibberellins GA4/7 according to Regulation (EC) No 1272/2008 is not required. The endpoint was agreed in the EFSA Conclusion and is still considered valid.

RMS comments and conclusion:

The two studies on acute inhalation toxicity were submitted during the previous EU review of GA4/7. The studies followed guidelines equivalent to OECD 403 and GLP principles. The nose-only exposure was used in the first study and in the second one the whole-body exposure. The concentrations tested in the studies were the maximum attainable concentrations. No major deviations has been observed in the first study compared to the OECD 403 (2009). The MMAD in the first study was >4 μm with SD >3 μm, thus exceeding the maximum MMAD and SD recommended in the current guideline OECD 403. However, a recalculations was done, giving an estimated exposure concentration for a MMAD of 4.0 μm 5.12 mg/L. The study is considered acceptable. In the second study, the MMAD was 5.83 μm at estimated exposure of 2.98 mg/L. No particle size distribution data were presented. In the second study the body weight observations are lacking for day 3. The study is considered to of limited acceptability, and is used for supplementary information.

The acute inhalation LC_{50} for rats (males, females, combined) is > 5 mg/L air 4h (nose only). No classification regarding acute inhalation toxicity is required for GA4/7 according to criteria laid down in Regulation (EC) No 1272/2008; the test substance is not subject to labelling requirements.

b) <u>Evaluation of additional data for the purpose of renewal of Annex I inclusion</u>

No additional data was submitted or requested for the purpose of renewal of approval of GA4/7.

B.6.2.4. Skin irritation

a) Previous evaluation (2005-2011)

Data on the *in vivo* skin irritation potential of GA4/7 were submitted during the EU review and are available in the EU DAR. The studies below were considered acceptable in the EFSA conclusion and are considered adequate for supporting renewal of GA4/7; no new *in vivo* skin irritation studies are submitted. Due to the availability of older acceptable *in vivo* studies, the tiered testing strategy set out in Regulation (EU) No 283/2013 does not apply, and therefore *in vitro* studies have not been performed. EFSA (2012) concluded that GA4/7 is not irritating to skin.

B.6.2.4/01

PREVIOUS EVALUATION	This study was evaluated in the DAR and has been considered by EFSA.
Data point addressed:	CA 5.2.4/01

	(B.6.2.1.4 DAR)
Author(s) (year):	[redacted] (1997)
Title:	GA4/7 99% technical: Skin irritation to the rabbit
Laboratory report / project number:	[redacted]
Testing facility:	[redacted]
Published:	No
Test guideline used:	OECD 404, EEC B.4, US EPA 152-14
Deviations:	The study was conducted to an earlier version of OECD 404 (a higher number of animals were used compared to current guidelines and a sequential testing strategy was not followed), however the study is acceptable for hazard classification
GLP:	Yes
EU Agreed Endpoint:	Non irritant

Executive summary

A primary dermal irritation study was conducted with 6 male New Zealand White rabbits and gibberellins GA4/7 (purity 99%; GA4 >90%, batch D105). An amount of 0.5 g test substance moistened with distilled water was applied to the shaved skin of each animal and covered with a semi-occlusive dressing for 4 hours. Skin reactions were evaluated according to the Draize scoring system at 1, 24, 48 and 72 hours after patch removal. No dermal response to treatment was observed in any animal during the observation period.

I Materials and Methods

A. Materials:

1. Test Material Gibberellins GA4/7

Description White powder

Lot/Batch D105

Purity GA4 >90%

Stability Assumed to be stable for the duration of the study

2. Vehicle Distilled water

3. Test animals Rabbits

Species New Zealand white

Age 11-13 weeks old

Weight 2.4 to 3.1 kg

Source [redacted]

Acclimation period Acclimation took place but time period not specified

Diet Standard Lab rabbit diet (SDS Stanrab (P) Rabbit Diet), *ad libitum*

Water Tap water *ad libitum*

Housing Animals were housed individually in plastic cages with perforated floors

4. Environmental conditions

Temperature	~19°C
Humidity	30-70%
Air changes	~19 air changes/hour
Photoperiod	12 hour light/dark cycle

B. Study Design and Methods

1. In life dates: 6 to 9 August 1996

2. Animal assignment and treatment

A group of 6 male rabbits were assigned to the study. Approximately 24 hours prior to test substance application, hair was removed from the dorso-lumbar region of each rabbit. A 0.5 g amount of the test substance was applied under a gauze pad that had been moistened with 0.5 mL distilled water onto one intact skin site. Each treatment site was covered with elastic adhesive dressing for 4 hours. At the end of the exposure period, the semi-occlusive dressing was removed and the treatment site washed with warm water and blotted dry with absorbent paper. All animals were observed daily for clinical signs of toxicity. Dermal reactions to treatment were evaluated approximately 60 minutes after dressing removal and at 24, 48 and 72 hours. The numerical Draize scoring system was used.

3. Statistics The data do not warrant statistical analysis

II Results and Discussion

A. Findings No signs of toxicity or ill health were noted in any rabbit during the observation period. No dermal response to treatment was observed in any animal throughout the observation period.

Table B 6.2.4/01-1 Individual and mean skin irritation scores according to Draize

	Erythema						**Oedema**					
Animal No.	221	222	223	224	225	226	221	222	223	224	225	226
After 60 minutes	0	0	0	0	0	0	0	0	0	0	0	0
After 24 hr	0	0	0	0	0	0	0	0	0	0	0	0
After 48 hr	0	0	0	0	0	0	0	0	0	0	0	0
After 72 hr	0	0	0	0	0	0	0	0	0	0	0	0
Mean score 24-72 h	0.0						0.0					

Conclusion

Gibberellins GA4/7 was not irritating to rabbit skin following a 4 hour semi-occlusive exposure. Classification according to Regulation (EC) No 1272/2008 is not required. The endpoint was agreed in the EFSA Conclusion and is still considered valid.

B.6.2.4/02

PREVIOUS EVALUATION	This study was evaluated in the DAR and has been considered by EFSA.

Data point addressed:	CA 5.2.4/02
Author(s) (year):	[redacted] (1988) (B.6.2.1.4 DAR)
Title:	Primary dermal irritation study of Gibberellins A4A7 (GA_4A_7) in rabbits
Laboratory report / project number:	[redacted]
Testing facility:	[redacted]
Published:	No
Test guideline used:	US EPA FIFRA Guideline 81-5, comparable to OECD 404
Deviations:	The study was conducted to US guideline; a higher number of animals were used compared to current guidelines and a sequential testing strategy was not followed, however the study is acceptable for hazard classification
GLP:	Yes
EU Agreed Endpoint:	Non irritant

Executive summary

A primary dermal irritation study was conducted with 6 New Zealand White rabbits (3/sex) and gibberellins GA4/7 (batch 16-213-CD). An amount of 0.5 g test substance moistened with 0.9% saline was applied to the shaved skin of each animal and covered with a semi-occlusive dressing for 4 hours. At the end of the exposure period the dressings were removed and the test site was washed with warm water. Skin reactions were evaluated according to the Draize scoring system at 30 minutes, 24, 48 and 72 hours after patch removal. No dermal response to treatment was observed in any animal during the observation period.

I Materials and Methods

A. Materials:

1. Test Material Gibberellins GA4/7

Description: White powder

Lot/Batch: 16-213-CD

Purity: Not stated in report but quoted as 90.0% for same batch in other reports

Stability: Assumed to be stable for the duration of the study

2. Vehicle 0.9% saline

3. Test animals Rabbits

Species: New Zealand white

Age: Young adult

Weight: 2468-2560 g males; 2398-2544 g females

Source: [redacted]

Acclimation period: 7 days

Diet: Standard Lab rabbit diet (Purina High Fiber Rabbit Chow #5326), measured amount

Water	Tap water *ad libitum*
Housing	Animals were housed individually in screen bottom cages

4. Environmental conditions

Temperature	~20-26°C
Humidity	58-69%
Air changes	Not stated
Photoperiod	Alternating 12 hour light and dark cycle

B. Study Design and Methods

1. In life dates: 6 to 9 July 1988

2. Animal assignment and treatment

A group of 6 rabbits (2 females and 4 males) were assigned to the study. Approximately 24 hours prior to test substance application, hair was clipped from the back and flanks of each animal. A 0.5 g amount of the test substance was moistened with 0.9% saline and applied onto one intact skin site. The treated area was covered with a gauze patch and semi-occlusive dressing. At the end of the 4 hour exposure period, the dressing was removed and the treatment site washed with warm water and disposable paper towels. Dermal reactions to treatment were evaluated approximately 30 minutes after dressing removal and at 24, 48 and 72 hours. The numerical Draize scoring system was used.

3. Statistics The data do not warrant statistical analysis

II Results and Discussion

A. Findings No dermal response to treatment was observed in any animal throughout the observation period.

Table B. 6.2.4/02-1 Individual and mean skin irritation scores according to Draize

	Erythema						**Oedema**					
Animal No. and sex	1M	2M	3M	1F	2F	3F	1M	2M	3M	1F	2F	3F
After 30 minutes	0	0	0	0	0	0	0	0	0	0	0	0
After 24 hr	0	0	0	0	0	0	0	0	0	0	0	0
After 48 hr	0	0	0	0	0	0	0	0	0	0	0	0
After 72 hr	0	0	0	0	0	0	0	0	0	0	0	0
Mean score 24-72 h	0.0						0.0					

Conclusion

Gibberellins GA4/7 was not irritating to rabbit skin following a 4 hour semi-occlusive exposure. Classification according to Regulation (EC) No 1272/2008 is not required. The endpoint was agreed in the EFSA Conclusion and is still considered valid.

RMS comments and conclusion:

The two studies on skin irritation were submitted during the previous EU review of GA4/7. The studies followed the OECD 404 (1981) or a guideline equivalent to it and GLP principles. The reported studies` deviations when compared to the latest version of the OECD 404 (2015) do not affect the outcome of the tests. Thus, the studies are found acceptable for hazard classification.

GA4/7 did not irritate skin of treated rabbits. No classification regarding skin irritation is required for GA4/7 according to criteria of Regulation 1272/2008.

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

No additional data was submitted or requested for the purpose of renewal of approval of GA4/7.

B.6.2.5. Eye irritation

Data on the *in vivo* eye irritation potential of GA4/7 were submitted during the EU review and are available in the EU DAR. The studies below were considered acceptable in the EFSA conclusion and are considered adequate for supporting renewal of GA4/7; no new *in vivo* eye irritation studies are submitted. Due to the availability of older acceptable *in vivo* studies, the tiered testing strategy set out in Regulation (EU) No 283/2013 does not apply, and therefore *in vitro* studies have not been performed. EFSA (2012) concluded that GA4/7 is a moderate eye irritant; classification as a Category 2 Eye Irritant is required according to Regulation (EC) No 1272/2008.

B.6.2.5/01

PREVIOUS EVALUATION	This study was evaluated in the DAR and has been considered by EFSA.
Data point addressed:	CA 5.2.5/01 (B.6.2.1.5 DAR)
Author(s) (year):	[redacted] (1997)
Title:	GA4/7 99% technical: Eye irritation to the rabbit
Laboratory report / project number:	[redacted]
Testing facility:	[redacted]
Published:	No
Test guideline used:	OECD 405, EEC Method B.5, US EPA 152-13
Deviations:	The study was conducted to an earlier version of OECD 405 (a higher number of animals was used compared to current guidelines and no analgesia was applied before test substance administration) however the study is acceptable for hazard classification
GLP:	Yes
EU Agreed Endpoint:	Moderate irritant

Executive summary

A primary eye irritation study was conducted with 6 New Zealand White rabbits and gibberellins GA4/7 (purity 99%; GA4 >90%, batch D105). A screening study was conducted with 1 rabbit initially; the test substance was instilled into the right conjunctival sac for 30 seconds, then the eye was washed for 30 seconds. A severe reaction was not observed therefore the study proceeded to the main test on the following day: the test substance (0.1 mL) was instilled into the right conjunctival sac of 6 rabbits. The eyes were not washed, and the left eye remained untreated to serve as the control. Eyes were evaluated for reactions according to the Draize scoring system at 1, 24, 48, 72 and 96 hours after instillation.

No corneal damage or iridial inflammation was observed in the screening study. A diffuse crimson colouration of the conjunctivae with slight swelling of the eyelids was seen. The eye was normal four days after instillation. In the main study, no corneal damage or iridial inflammation was observed. A diffuse crimson colouration of the conjunctivae was seen in all animals one hour after instillation, accompanied in three animals by swelling with partial eversion of eyelids and in all animals by conjunctival discharge. The reactions gradually ameliorated and the eyes were normal at three or four days after instillation.

I Materials and Methods

A. Materials:

1.	**Test Material**	Gibberellins GA4/7
	Description	White powder
	Lot/Batch	D105
	Purity	GA4 >90%
	Stability	Assumed to be stable for the duration of the study
2.	**Vehicle**	None
3.	**Test animals**	Rabbits
	Species	New Zealand white
	Age	12-18 weeks old
	Weight	2.7 to 3.9 kg
	Source	[redacted]
	Acclimation period	Acclimation took place but time period not specified
	Diet	Standard Lab rabbit diet (SDS Stanrab (P) Rabbit Diet), *ad libitum*
	Water	Tap water *ad libitum*
	Housing	Animals were housed individually
4.	**Environmental conditions**	
	Temperature	19°C
	Humidity	30-70%
	Air changes	19 air changes/hour
	Photoperiod	12 hour light/dark cycle

B. Study Design and Methods

1. In life dates: 12 to 19 August 1996

2. Animal assignment and treatment

Seven male rabbits were assigned to the study. The test substance (0.1 mL) was instilled into the everted lid of one eye of each animal (the contralateral eye remained untreated). The eyelids were then gently held together for one

second before releasing. One animal was treated in advance of the others, and the eye washed 30 seconds after instillation for half a minute to ensure that if a severe response were produced, no further animals would be exposed. One further pilot animal was treated in advance of the other five (eyes remained unwashed). The remaining five animals were then treated and the eyes left unwashed. All animals were observed daily for signs of ill health or toxicity. Examination of both eyes was made after 1 hour and 1, 2, 3 (equivalent to 24, 48 and 72 hours post dosing) days after installation and irritation scored according to the Draize system. An additional observation was made for one animal 4 days after treatment. Observation of the eyes was aided by the use of a handheld light.

3. Statistics The data do not warrant statistical analysis

II Results and Discussion

A. Findings No signs of toxicity or ill health were seen in any rabbit during the observation period. No corneal damage or iridial inflammation was noted in the pilot eye (rinsed eye). A diffused crimson colouration of the conjunctivae with slight swelling of the eyelids was observed in this animal; the eye was normal four days after instillation. In the main study, all animals were considered to give a 'positive' response. No corneal damage or iridial inflammation was noted. A diffuse crimson colouration of the conjunctivae was seen in all animals one hour after instillation, accompanied in three animals by swelling with partial eversion of eyelids and in all animals by discharge. The reactions gradually ameliorated and the eyes were normal three ti four days after instillation.

Table B 6.2.5/01-1 Eye irritation scores according to the Draize scheme-pilot animal

Rabbit No.	Region of eye		Observation time point: 1 h	Day after instillation: 1	2	2	4	Mean score 24-72 h (days 1-3)
165*#	Cornea	Opacity	0	0	0	0	0	0
	Iris		0	0	0	0	0	0
	Conjunctiva	Redness	2	1	1	1	0	1
		Chemosis	1	0	0	0	0	0
		Discharge	3	0	0	0	0	-

** Eye washed after 30 seconds # Pilot animal*

-not used for classification according to CLP so mean score not provided

Table B.6.2.5/02-2 Eye irritation scores according to the Draize scheme-main test (unrinsed eyes)

Rabbit No.	Region of eye		Observation time point: 1 h	Day after instillation: 1	2	2	4	Mean score 24-72 h (days 1-3)
166#	Cornea	Opacity	0	0	0	0	0	0
	Iris		0	0	0	0	0	0
	Conjunctiva	Redness	2	2	1	1	0	1.33
		Chemosis	2	1	1	0	0	0.67
		Discharge	3	1	0	0	0	-
167	Cornea	Opacity	0	0	0	0		0

	Iris		0	0	0	0		0
	Conjunctiva	Redness	2	2	2	0		1.33
		Chemosis	2	2	1	0		1
		Discharge	1	0	0	0		-
168	Cornea	Opacity	0	0	0	0		0
	Iris		0	0	0	0		0
	Conjunctiva	Redness	2	2	1	0		1
		Chemosis	2	1	1	0		0.67
		Discharge	1	1	0	0		-
169	Cornea	Opacity	0	0	0	0		0
	Iris		0	0	0	0		0
	Conjunctiva	Redness	2	1	1	0		0.67
		Chemosis	1	1	0	0		0.33
		Discharge	1	0	0	0		-
170	Cornea	Opacity	0	0	0	0		0
	Iris		0	0	0	0		0
	Conjunctiva	Redness	2	2	1	0		1
		Chemosis	1	1	1	0		0.67
		Discharge	2	1	0	0		-
171	Cornea	Opacity	0	0	0	0		0
	Iris		0	0	0	0		0
	Conjunctiva	Redness	2	2	2	0		1.33
		Chemosis	1	1	1	0		0.67
		Discharge	2	1	0	0		-

– not used for classification according to CLP so mean score not provided, # Pilot animal

 # - pilot animal

Conclusion

Gibberellins GA4/7 caused irritation to the rabbit eye; however mean scores at 24, 48 and 72 hours were not sufficient to trigger classification according to Regulation (EC) No 1272/2008. The endpoint (moderate eye irritant) was agreed in the EFSA Conclusion and is still considered valid.

B.6.2.5/02

PREVIOUS EVALUATION	This study was evaluated in the DAR and has been considered by EFSA.
Data point addressed:	CA 5.2.5/02
Author(s) (year):	[redacted] (1988) (B.6.2.1.5 DAR)

Title:	Primary eye irritation study of Gibberellins A4A7 (GA_4A_7) in rabbits
Laboratory report / project number:	[redacted]
Testing facility:	[redacted]
Published:	No
Test guideline used:	US EPA FIFRA Guideline 81-4, comparable to OECD 405
Deviations:	A higher number of animals was used compared to current guidelines, no analgesia was applied before test substance administration and a sequential testing strategy was not followed, however the study is acceptable for hazard classification
GLP:	Yes
EU Agreed Endpoint:	Moderate irritant

Executive summary

A primary eye irritation study was conducted with 6 New Zealand White rabbits and gibberellins GA4/7 (batch 16-213-CD). The test substance (0.1 mL equivalent to 60 mg) was instilled into the conjunctival sac of each animal. The eyes were not washed, and the contralateral eye remained untreated to serve as the control. Eyes were evaluated for reactions according to the Draize scoring system at 1, 24, 48, 72, and 96 hours, and 7 days after instillation. At the 72 hour and Day 7 assessments the eyes were also examined by use of sodium fluorescein to determine the presence and extent of any corneal epithelial damage. The test substance produced iridial involvement and moderate to severe conjunctival irritation when instilled into the eyes of six albino rabbits. A slight corneal opacity was also observed in one animal at the 24-hour observation only. All ocular irritation cleared within 7 days of instillation of the test substance.

I Materials and Methods

A. Materials:

1. Test Material Gibberellins GA4A7

Description: White powder

Lot/Batch: 16-213-CD

Purity: Not stated in report but quoted as 90.0% for same batch in other reports

Stability: Assumed to be stable for the duration of the study

2. Vehicle None

3. Test animals Rabbits

Species: New Zealand White Hra: (NZW)SPF

Age: Young adult

Weight: 2236-2328 g males; 2272-2376 g females

Source: [redacted]

Acclimation period: 7 days

Diet: Standard Lab rabbit diet (High Fiber Rabbit Chow 5326, Purina Mills, Inc.); measured amount

Water: Tap water *ad libitum*

Housing: Animals were housed individually in screen bottom cages

4. Environmental conditions

Temperature	~20-26°C
Humidity	59-74%
Air changes	Not stated
Photoperiod	Not stated

B. Study Design and Methods

1. In life dates: 8-15 July 1988

2. Animal assignment and treatment

Six rabbits were selected, three males and three females. The test material was presented as a powder and so the weight equivalent of 0.1 mL was instilled. The bulk density was determined and a weight of 60 mg was administered. The dose was instilled into the lower conjunctival sac of one eye of each rabbit, with the contralateral eye serving as the untreated control in each instance. Upper and lower lids were held closed briefly and then released. Any initial pain response was recorded. The eyes were not flushed to remove any residual test material. Treated eyes were observed 1, 24, 48, 72 and 96 hours post-dosing and at day 7 after instillation and reactions assessed according to Draize. At the 72 hour and Day 7 assessments the eyes were also examined by use of sodium fluorescein to determine the presence and extent of any corneal epithelial damage. The animals were weighed at termination, when they were discarded without necropsy.

3. Statistics The data do not warrant statistical analysis

II Results and Discussion

A. Findings

The rabbits all showed an initial response consisting of excessive pawing at the treated eye following instillation of the dose. Blanching of the conjunctivae was apparent for all six rabbits at 1 and 24 hours, for 4 rabbits at 48 hours and one rabbit only at 72 and 96 hours. Conjunctival haemorrhage (described in report as petite haemorrhage) was apparent for two rabbits one hour after dosing and for one rabbit at 24 hours. Scattered diffuse corneal opacity (affecting less than a quarter of the ocular orbit) was noted for one rabbit at 24 hours post-dosing only. Sodium fluorescein dye applied at 72 hours and at day 7 revealed no corneal damage. Iridial congestion (Draize score of 1) was apparent for all rabbits at 1 hour and for two rabbits at 24 hours post-dosing but these reactions had fully resolved within 48 hours. All treated eyes produced a significant amount of clear conjunctival discharge within an hour of dosing but the response had generally resolved within 48 hours. Conjunctival reactions within an hour of dosing resulted in a diffuse beefy redness for all six treated eyes. Some amelioration was apparent in each eye over the following four days but all eyes remained injected or showing a crimson coloration at 96 hours. Conjunctival redness was accompanied by slight to obvious swelling (Draize score of 1 or 2) for between 48 and 96 hours following instillation of gibberellins GA4A7. All reactions had resolved by Day 7 when all eyes appeared overtly normal.

Table CA 5.2.5-4 Eye irritation scores according to the Draize scheme

Rabbit No. and sex	Region of eye	Observation time point (hours)						Mean score 24-72 h
		1	24	48	72	96	168	

Rabbit No. and sex	Region of eye		Observation time point (hours)						Mean score 24-72 h
			1	24	48	72	96	168	
1M	Cornea	Opacity	0	0	0	0	0	0	0
	Iris		1	1	0	0	0	0	0.33
	Conjunctiva	Redness	3	2	2	1	1	0	1.67
		Chemosis	2	2	1	1	0	0	1.33
		Discharge	3	0	0	0	0	0	-
2M	Cornea	Opacity	0	0	0	0	0	0	0
	Iris		1	0	0	0	0	0	0
		Redness	3	2	2	2	2	0	2
		Chemosis	2	1	1	1	1	0	1
		Discharge	1	0	0	0	0	0	-
3M	Cornea	Opacity	0	0	0	0	0	0	0
	Iris		1	1	0	0	0	0	0.33
	Conjunctiva	Redness	3	3	2	2	2	0	2.33
		Chemosis	2	2	1	1	1	0	1.33
		Discharge	2	2	0	0	1	0	-
4F	Cornea	Opacity	0	0	0	0	0	0	0
	Iris		1	0	0	0	0	0	0
	Conjunctiva	Redness	3	2	2	2	2	0	2
		Chemosis	2	2	1	1	1	0	1.33
		Discharge	3	0	0	0	0	0	-
5F	Cornea	Opacity	0	1	0	0	0	0	0.33
	Iris		1	0	0	0	0	0	0
	Conjunctiva	Redness	3	2	2	2	2	0	2
		Chemosis	2	2	1	0	0	0	1
		Discharge	2	1	1	0	0	0	-
6F	Cornea	Opacity	0	0	0	0	0	0	0
	Iris		1	0	0	0	0	0	0
	Conjunctiva	Redness	3	2	2	1	1	0	1.67
		Chemosis	2	2	1	0	0	0	1
		Discharge	2	2	0	0	0	0	-

– not used for classification according to CLP so mean score not provided

Conclusion

Gibberellins GA4/7 caused irritation to the rabbit eye, based on the mean scores at 24, 48 and 72 hours classification as a Category 2 Eye Irritant is required according to Regulation (EC) No 1272/2008. The endpoint (moderate eye irritant) was agreed in the EFSA Conclusion and is still considered valid.

RMS comments and conclusion:

The two studies on eye irritation were submitted during the previous EU review of GA4/7. The purity of the tested material in the second study was not stated, however, it is assumed to be 90.0% GA4/7 based on the information from some other study reports where the same batch was tested. The studies followed the OECD 405 (1981) or a guideline equivalent to it and principles of GLP. The studies` deviations already noted by the applicant, even compared to the latest version of the OECD 405 (2017), do not affect the outcome of the tests. The studies are acceptable.

GA4/7 moderately irritated eyes of treated rabbits, as indicated by conjunctival redness and chemosis observed in all the studies. Based on the mean scores at 24, 48 and 72 hours, conjunctival redness of grade ≥2 was observed in 4/6 rabbits. Thus, gibberellins (GA4/7) needs to be classification as Eye Irrit. 2, H319 according to criteria of Regulation 1272/2008.

b) <u>Evaluation of additional data for the purpose of renewal of Annex I inclusion</u>

No additional data was submitted or requested for the purpose of renewal of approval of GA4/7.

B.6.2.6. Skin sensitization

Data on the skin sensitisation potential of GA4/7 were submitted during the EU review and are available in the EU DAR. The studies were considered acceptable in the EFSA conclusion and are considered adequate for supporting renewal of GA4/7; no new sensitisation studies are submitted. EFSA (2012) concluded that GA4/7 is non-sensitising based on a Magnusson and Kligman (M & K) guinea pig maximisation test.
Regulation (EU) No 283/2013 states that the preferred method for testing is the Local Lymph Node Assay, but where a guinea pig study that meets OECD guidelines and provides a clear result is available, further testing is not required on animal welfare grounds. A clear result was obtained in the most recent study conducted to current guidelines (CA 6.2.6/01) therefore no further testing is considered necessary.

B.6.2.6/01

PREVIOUS EVALUATION	This study was evaluated in the DAR and has been considered by EFSA.
Data point addressed:	CA 5.2.6/01 (B.6.2.1.6 DAR)
Author(s) (year):	[redacted] (1997)
Title:	GA4/7 99% technical: Skin sensitisation in the guinea pig
Laboratory report / project number:	[redacted]
Testing facility:	[redacted]
Published:	No
Test guideline used:	OECD 406, US EPA FIFRA 81-6, EEC Method B.6
Deviations:	None
GLP:	Yes
EU Agreed Endpoint:	Non-sensitising (M & K)

Executive summary

A dermal sensitisation study was conducted with gibberellins GA4/7 technical (purity 99%; GA4 >90%, batch D105) and Dunkin-Hartley guinea pigs. An irritation screen was conducted to confirm dose levels for the main study (2.5% for intradermal induction, 70% for epidermal induction, 35% and 70% at challenge). Induction was performed by intradermal injections of Freund's Complete Adjuvant (maximization test) and the test substance (Day 1), then topical application of the test substance for 48 hours (Day 8). Challenge was performed for 24 hours two weeks after the second sensitization, and the sites were examined 24 and 48 hours after removal of the patches. Necrosis and slight irritation was noted on the induction site in both the test and control animals. Following challenge with a 70% and 35% preparation of the test substance in acetone, no dermal reactions were seen in any of the test or control animals. Gibberellins GA4/GA7 did not produce evidence of skin sensitisation in any of the twenty test animals.

I Materials and Methods

A. Materials:

1.	**Test Material**	Gibberellins (GA4/7 technical)
	Description	White powder
	Lot/Batch	D105
	Purity	GA4 >90%
	Stability	Assumed to be stable for the duration of the study
2.	**Vehicle and/or positive control**	5% acetone in Alembicol D, Freund's Complete Adjuvant emulsion, hexyl cinnamic aldehyde, mercaptobenzothiazole and benzocaine
3.	**Test animals**	Guinea pigs
	Species	Dunkin/Hartley
	Age	4-5 weeks old
	Weight	301-345 g
	Source	[redacted]
	Acclimation period	12 days
	Diet	Vitamin C enriched guinea pig diet (FD1), *ad libitum.* Hay given weekly
	Water	Tap water *ad libitum*
	Housing	Animals were housed in groups of 5 in suspended metal cages with wire mesh floors
4.	**Environmental conditions**	
	Temperature	21°C
	Humidity	30-70%
	Air changes	15 air changes/hour
	Photoperiod	12 hour light/dark cycle

B. Study Design and Methods

1. In life dates: 17 July to 16 August 1996

2. Animal assignment and treatment

A preliminary study was conducted with six animals to determine the intradermal and topical irritancy for a range of dilutions, in order to identify concentrations that would produce irritation suitable for the induction phase and a maximum non-irritant concentration for topical challenge. 70% w/v in acetone was found to be the maximum non-

irritant concentration. Based on the preliminary study, 2.5% and 5% w/v in % acetone in Alembicol D was used for intradermal induction injections, and 70% w/v in acetone was used for topical induction. 70 and 35% w/v in acetone were used for topical challenge.

Ten control animals and twenty test animals were assigned to the main study. The treatment regime involved an initial induction exposure by intradermal injection on day 1, a second induction exposure by topical administration on day 8 and challenge by topical administration on day 22. The test levels for dermal and intradermal inductions and challenge were selected following preliminary irritancy testing. A 40 x 60 mm area of dorsal skin on the scapular region of the guinea pig was clipped free of hair with electric clippers. Three pairs of intradermal injections were made into a 20 x 40 mm area within the clipped area. Injectables for the test animals were prepared as follows:

1. Freund's complete adjuvant was diluted with an equal volume of water for irrigation.
2. Gibberellins (GA4/7 99% technical), 2.5% w/v in 5% acetone in Alembicol D.
3. Gibberellins (GA4/7 99% technical), 2.5% w/v in a 50:50 mixture of Freund's complete adjuvant and 5% acetone in Alembicol D.

Six days after the injections, the same 40 x 60 mm interscapular area was clipped and shaved free of hair and the site was pretreated by gentle rubbing with 0.2 ml/site of 10% w/w sodium lauryl sulphate in petrolatum. Twenty-four hours later a 20 x 40 mm patch of Whatman No. 3 paper was saturated with approximately 0.4 mL of gibberellins (GA4/7 99% technical), 70% w/v in acetone. The patch was placed on the skin of the test animals and covered by a length of impermeable plastic adhesive. This was secured by elastic adhesive bandage and left in place for 48 hours. During the induction phase, the control animals were treated similarly to the test animals except that the test substance was omitted from the intradermal injections and topical application. The control and test animals were challenged topically two weeks after the topical induction application using gibberellins (GA4/7 99% technical) 70 and 35% w/v in acetone. Hair was removed from the left flank of each guinea pig. A 20 x 20 mm patch of Whatman No. 3 paper was saturated with approximately 0.2 mL of gibberellins (GA4/7 99% technical), 70% w/v in acetone and applied to an anterior site on the flank. Gibberellins (GA4/7 99% technical), 35% w/v in acetone was applied in a similar manner to a posterior site. The patches were sealed to the flank for 24 hours.

3. Statistics The data do not warrant statistical analysis

II Results and Discussion

A.	Clinical signs	No clinical signs of ill health or toxicity were noted during the study.
B.	Body weight	Body weights increased during the study.
C.	Induction	Intradermal injections: Necrosis was recorded at sites receiving Freund's Complete Adjuvant in test and control animals. Slight irritation was seen in test animals at sites receiving gibberellins (GA4/7 99% technical), 2.5% w/v in 5% acetone in Alembicol D and slight irritation was observed in control animals receiving 5% acetone in Alembicol D alone. Necrosis was recorded at sites receiving (50:50) Freund's Complete Adjuvant and gibberellins (GA4/7 99% technical), 2.5% w/v in 5% acetone in Alembicol D in both the test and control animals. Topical application: Slight erythema was observed in test animals following topical application with gibberellins (GA4/7 99% technical), 70% w/v in acetone. Slight erythema was seen in the control guinea pigs.
D.	Challenge	There were no dermal reactions seen in any of the test or control animals; all scored were zero.
E.	Positive control	The positive control tests conducted regularly at the test facility gave a strong positive result, confirming the validity of the assay.

Conclusion

Gibberellins GA4/7 did not show sensitizing potential in a maximization test. Classification according to Regulation (EC) No 1272/2008 is not required. The endpoint was agreed in the EFSA Conclusion and is still considered valid.

B.6.2.6/02

PREVIOUS EVALUATION	This study was evaluated in the DAR and has been considered by EFSA.
Data point addressed:	CA 5.2.6/02 (B.6.2.1.6 DAR)
Author(s) (year):	[redacted]. (1988)
Title:	Dermal sensitization study of Gibberellins A4A7 (GA_4A_7) in guinea pigs (Maximization test)
Laboratory report / project number:	[redacted]
Testing facility:	[redacted]
Published:	No
Test guideline used:	US EPA FIFRA Guideline 81-5, comparable to OECD 406
Deviations:	None
GLP:	Yes
EU Agreed Endpoint:	Non-sensitising [M&K]

Executive summary

A dermal sensitisation study was conducted with gibberellins GA4/7 (batch 16-213-CD) and Dunkin/Hartley albino guinea pigs. An irritation screen was conducted to confirm dose levels for the main study (25% for induction and challenge). Induction was performed by intradermal injections of Freund's Complete Adjuvant (maximization test) and the test substance (day 1), then topical application of the test substance for 48 hours (day 8). Challenge was performed for 24 hours two weeks after the second induction, and the sites were examined 24 and 48 hours after removal of the patches. A slight dermal reaction to the challenge application of the test substance was observed in one test animal at 24 hours. None of the negative control animals exhibited a dermal reaction at challenge.

I Materials and Methods

A. Materials:

1.	**Test Material**	Gibberellins GA4/7
	Description	White powder
	Lot/Batch	16-213-CD
	Purity	Not stated in report but quoted as 90.0% for same batch in other reports
	Stability	Assumed to be stable for the duration of the study
2.	**Vehicle and/or positive control**	Sterile water for injection and Freund's Complete Adjuvant (FCA)

3.	**Test animals**	Guinea pigs
	Species	Dunkin/Hartley Hra: (DH)SPF
	Age	Young adults
	Weight	432-576 g
	Source	[redacted]
	Acclimation period	7 days
	Diet	Purina Certified Guinea Pig Chow 5026, *ad libitum*
	Water	Tap water *ad libitum*
	Housing	Animals were individually housed in screen-bottomed suspended stainless steel cages

4. Environmental conditions

Temperature	19-31°C
Humidity	53-77%
Air changes	Not stated
Photoperiod	Alternating 12 hour light and dark cycle

B. Study Design and Methods

1. In life dates: 6 to 30 July 1998

2. Animal assignment and treatment

Following a range finding preliminary study in which 4 animals were dosed by topical application of four dose formulations, 1, 10, 15 and 25% w/w preparations in petrolatum, to determine a moderately irritating dose for the topical induction phase and a non-irritating dose for use in the challenge phase, suitable doses were chosen for the main study. Two groups of twenty male guinea pigs were allocated to the study. The test group received the test substance in the intradermal and topical induction phases but the control group was untreated during the induction phase. Gibberellins GA4/7 was mixed with sterile water or with FCA plus sterile water, to a final concentration of 5% w/v, and injected at paired injection sites in the interscapular region of the test group on Day 1. The dose volume injected was 0.05 mL/site rather than 0.1 mL/injection as required by the test guidelines. On Day 7 the test group animals were treated by topical application of sodium lauryl sulphate (10% w/w suspension in petrolatum) over the scapular region bounded by the intradermal injection sites. On Day 8 a 25% w/w suspension of gibberellins GA4/7 in petrolatum was applied over the same area and covered by an occlusive dressing that remained in place for 48 hours. The control animals were not dosed in this phase. Two weeks later all animals were dosed by application of a 25% w/w preparation of gibberellins GA4/7 in petrolatum to the clipped/shaved right flank. The dose was covered by an even layer of Whatman No 2 filter paper secured for 24 hours by an occlusive dressing. After completion of the exposure period the dressings were removed and test sites wiped clean. Approximately 21 hours later the flanks were closely shaved to remove re-grown hair and enable sites to be accurately read. Assessments, according to modified Draize scoring system, were completed 24 and 48 hours after removal of the dressing. The animals were observed for clinical signs of toxicity throughout the study and body weights were recorded prior to first dosing occasion and then at weekly intervals through the study. Guinea pigs were terminated on Day 25 after completion of the challenge assessments and discarded without necropsy.

3. Statistics The data do not warrant statistical analysis

II Results and Discussion

A. Clinical signs and mortality — One test group animal had brown staining of the abdomen on Days 10, 11 and 12, was ataxic on Day 12 and was found dead on Day 13. Gross necropsy revealed no

		macroscopic abnormalities and the cause of death was not established. Four test group animals were hypoactive on day 10 and three similarly on day 11 and 12, with only one hypoactive animal on day 13. The remaining animals appeared clinically normal throughout the study.
B.	Dermal reactions	A very slight erythematous reaction was noted in one test group animal 24 hours after challenge. No other dermal reactions were observed for the test or control groups. The incidence of reaction (5%) was considerably lower than the EC threshold of 30% for considering gibberellins GA4/7 to be a contact sensitiser.

Conclusion

Gibberellins GA4/7 showed a very slight sensitising potential in a maximization test, however responses were not sufficient to trigger classification according to Regulation (EC) No 1272/2008. The endpoint was agreed in the EFSA Conclusion and is still considered valid.

B.6.2.6/03

PREVIOUS EVALUATION	This study was evaluated in the DAR and has been considered by EFSA.
Data point addressed:	CA 5.2.6/03 (B.6.2.1.6 DAR)
Author(s) (year):	[redacted] (1994)
Title:	Gibberellin A4/A7: Skin sensitization to the guinea pig
Laboratory report / project number:	[redacted]
Testing facility:	[redacted]
Published:	No
Test guideline used:	In-house method (maximisation test) comparable with OECD 406
Deviations:	Not applicable
GLP:	Yes
EU Agreed Endpoint:	Non-sensitising [M&K]

Executive summary

A dermal sensitisation study was conducted with gibberellins GA4/7 (purity 90.2%, batch no P2) and albino guinea pigs. Induction was performed by intradermal injections of Freund's Complete Adjuvant (maximization test) and the test substance (day 1), then topical application of the test substance for 48 hours (day 8). Challenge was performed for 24 hours two weeks after the second sensitization, and the sites were examined 24 and 48 hours after removal of the patches. A sighting study was performed prior to the main study. Following challenge with a 30% preparation of the test substance in corn oil, scattered mild redness or moderate diffuse redness was seen in six of the twenty test animals. There were no erythematous reactions in any of the control animals. The net percentage response was calculated to be 30%. Following challenge with a 75%, 10% or a 3% w/v preparation of the test substance in corn oil, there were no erythematous reactions in any of the test or control animals. The net percentage response was calculated to be 0%.

I Materials and Methods

A. Materials:

1. Test Material	Gibberellins GA4/7
Description	White solid
Lot/Batch	P2
Purity	GA4/7: 90.2%
Stability	Assumed to be stable for the duration of the study
2. Vehicle and/or positive control	Corn oil and Freund's Complete Adjuvant (FCA), 2-mercaptobenzothiazole
3. Test animals	Guinea pigs
Species	Hsd/POC:DH
Age	Young adults
Weight	367-489 g main study females; 452-574 g positive control females
Source	[redacted]
Acclimation period	6 days
Diet	Labsure RGP Diet, *ad libitum*
Water	Tap water *ad libitum*
Housing	Animals were individually housed in screen-bottomed suspended stainless steel cages
4. Environmental conditions	
Temperature	15-19°C
Humidity	40-70%
Air changes	25-30/hour
Photoperiod	Alternating 12 hour light and dark cycle

B. Study Design and Methods

1. In life dates: June/July 1994 (main study)

2. Animal assignment and treatment

A preliminary study was completed to determine suitable dose levels for the intradermal injection and topical application aspects of the induction phase and non-irritant dose levels for use in the challenge phase. The preliminary study had three phases each containing 2 or 4 guinea pigs. The test material was prepared at up to 3% w/v in corn oil for intradermal injection to determine the highest level that could be tolerated locally and systemically. For topical induction the test material was prepared in corn oil to determine the highest concentration not eliciting reactions that exceeded moderate erythema or oedema in guinea pigs pre-treated with Freund's Complete Adjuvant (FCA) at least 14 days earlier. Lastly, further corn oil preparations were used to determine the highest non-irritant concentration, in guinea pigs pre-treated with FCA at least 14 days earlier, which could be applied in the challenge phase. The results of these preliminary investigations are not presented in the study report.

Thirty female guinea pigs were allocated to two groups in the main study, a test group of 20 animals and a control group containing 10 guinea pigs. The test regimen is set out in Table B.6.2.6/03-1 below. The test substance was injected into the clipped scapular region in three pairs of injection sites as formulations in corn oil or corn oil and FCA. The controls were injected with corn oil or corn oil/FCA alone. One week later the same region was clipped free of hair and a filter paper patch loaded with a 75% w/w preparation in corn oil was applied to the test group and a similar patch with corn oil alone was applied to the controls. The patches were covered by surgical tape and held in

place for 48 hours by an adhesive bandage. The dermal response at the topical induction sites was recorded 24 hours after bandage removal. Two weeks later both flanks of all animals were clipped free of hair and two filter paper patches backed by a rubber sheet were applied to each flank. The test substance formulations applied to each filter paper were:

distal left site: 75% w/v in corn oil
caudal left site: 30% w/v in corn oil
distal right site: 10% w/v in corn oil
caudal right site: 3% w/v in corn oil.

The patches and rubber sheet were held in place for 24 hours by means of an adhesive bandage. After completion of the exposure period the dressings were removed and the location of the application sites marked on each flank. Approximately 24 and 48 hours the reactions were assessed using a modified Draize scoring scale and the percentage of responders in the control group was subtracted from test group responders to quantify the sensitisation response. Guinea pigs were terminated on Day 25 after completion of the challenge assessments and discarded without necropsy.

The positive control study was conducted according to the same procedures except 2 mercaptobenzothiazole replaced the test material. The doses used were 3% w/v in corn oil for intradermal injection, 75% w/v for topical induction application and 30% w/v in corn oil for the challenge application.

3. Statistics The data do not warrant statistical analysis

II Results and Discussion

A. Clinical signs and mortality — None of the animals died. No clinical signs of reaction to treatment were reported. Body weight changes were not affected by the treatment regimen.

B. Dermal reactions — There were no reactions in the control group at any of the four concentrations tested or at any time point during the challenge assessment phase. There were no reactions in the test group at the three challenge sites treated with 3, 10 or 75% preparations of gibberellins GA4/7 in corn oil. The sites treated with the 30% formulation, for six of the twenty guinea pigs did develop some degree of reaction 48 hours after challenge. Four of these animals had only scattered mild redness and the other two had more pronounced, moderate redness. The report author interprets these results as indicating a 30% positive response to challenge at a single concentration. The six-point grading scheme for describing sensitising potential based on the percentage of positive responses indicates that 29-64% positive responses indicate a moderate sensitising potential or Grade 3. However, the results at 30% are not substantiated by the responses at lower concentrations - the 10 and 3% formulations elicited no reactions in any of the animals. The maximised exposure, 75%, also provides no evidence of sensitising potential.

This is a maximisation study design and yet the highest dose level did not induce sensitisation. Low concentrations of gibberellins GA4A7 also failed to induce sensitisation and it is doubtful whether the intermediate concentration (30%) that elicited a response is an accurate representation of sensitising potential. The 30% threshold was not exceeded at any of the tested concentrations, so at most there is a possible indication of weak skin sensitisation potential at specific doses.

C. Positive control — The positive control study provided a 95% positive response to application of 30% 2-mercaptobenzothiazole, demonstrating the sensitivity and appropriateness of the methods used in this study for detecting contact sensitisers.

Table B.6.2.6/03-1 Individual dermal reactions following topical challenge application of gibberellins GA4/7

Challenge dose	**75%**		**30%**		**10%**		**3%**	
Assessment time	**24 hr**	**48 hr**	**24 hr**	**48 hr**	**24 hr**	**48 hr**	**24 hr**	**48 hr**
Test group								
33	0	0	0	0	0	0	0	0
34	0	0	0	0	0	0	0	0
35	0	0	0	0	0	0	0	0
36	0	0	0	0	0	0	0	0
37	0	0	0	1	0	0	0	0
38	0	0	0	1	0	0	0	0
39	0	0	0	0	0	0	0	0
40	0	0	0	2	0	0	0	0
41	0	0	0	1	0	0	0	0
42	0	0	0	0	0	0	0	0
43	0	0	0	0	0	0	0	0
44	0	0	0	1	0	0	0	0
45	0	0	0	0	0	0	0	0
46	0	0	0	0	0	0	0	0
47	0	0	0	0	0	0	0	0
48	0	0	0	0	0	0	0	0
49	0	0	0	0	0	0	0	0
50	0	0	0	0	0	0	0	0
51	0	0	0	0	0	0	0	0
52	0	0	0	2	0	0	0	0
Control group								
53	0	0	0	0	0	0	0	0
54	0	0	0	0	0	0	0	0
55	0	0	0	0	0	0	0	0
56	0	0	0	0	0	0	0	0
57	0	0	0	0	0	0	0	0
58	0	0	0	0	0	0	0	0
59	0	0	0	0	0	0	0	0
60	0	0	0	0	0	0	0	0
61	0	0	0	0	0	0	0	0
62	0	0	0	0	0	0	0	0

0 *No reaction.*

1 *Scattered mild redness.*

2 *Moderate diffuse redness*

Conclusion

Gibberellins GA4/7 showed slight sensitizing potential in the maximization test, however the response was only observed at a challenge concentration of 30%, and not at 75%, 10% or 3%. Based on the weight of evidence, classification according to Regulation (EC) No 1272/2008 is not required. The endpoint was agreed in the EFSA Conclusion and is still considered valid.

RMS comments and conclusion:

Three studies were submitted, all using the M&K method. The studies followed OECD 406 (1992) or guideline comparable to OECD 406 and principles of GLP. The GA4// purity in three experiments was > 90 %. All three studies encountered minor deviations as not reporting numerical values of dermal effects seen after induction. However, the first study (B.6.2.6/01) is judged to be the most reliable. The effects after induction were described as slight irritation, slight erythema and necrosis in groups of control and test animals. No signs of skin irritation were observed after the challenging. Summary of positive control data were reported and judged to be adequate. In the second study (B.6.2.6/02) no information is given on the skin reactions after induction and no data on positive control were reported or mentioned in the study report. Thus, the second study (B.6.2.6/02) is considered as supportive. In the third study (B.6.2.6/03) no information is given on the skin reactions after induction. Summary of positive control data were reported and judged to be adequate. After challenging, 30% animals exhibited skin reaction in the tested mid group (30%). However, no skin reactions were observed at the highest (75%) and lower (3,% 10%) tested concertation's, thus the study results are judged equivocal.

Based on the all evaluated data for skin sensitisation no classification is required regarding skin sensitization according to criteria laid down in Regulation 1272/2008 as amended.

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

No additional data was submitted or requested for the purpose of renewal of approval of GA4/7.

B.6.2.7. Phototoxicity

a) Previous evaluation (2005-2011)

Phototoxicity study is a new data requirement. The study was not submitted for the first inclusion of GA4/7.

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

A study of phototoxicity is provided, based on the ultraviolet / visible light absorption properties of the active substance and the new data requirement in Regulation 283/2013.

PREVIOUS EVALUATION	Not previously evaluated
Data point addressed:	CA 5.2.7/01
Author(s) (year):	Gerbeix, C. (2018)
Title:	Gibberellic Acid A4/A7: 3T3 NRU Phototoxicity Assay
Laboratory report / project number:	Company study no. 45159 TIP
Testing facility:	CiToxLab, Evreux, France
Published:	No
Test guideline used:	OECD 432 (2004)
Deviations:	None
GLP:	Yes (certified laboratory)

Executive Summary

Gibberellic Acid A4/A7 (gibberellins GA4/7) was assayed for phototoxicity in cultured BALB/c 3T3 fibroblasts using the Neutral Red Uptake assay in two independent experiments. BALB/c 3T3 fibroblast cells seeded into 96-well microtitre plates were treated with a range of concentrations of Gibberellic Acid A4/A7 (0.32, 1, 3.17,

10.03, 31.69, 100.15, 316.46, 1000 µg/mL in the preliminary test or 263.34, 318.64, 385.55, 466.51, 564.48, 683.02, 826.45, 1000 µg/mL in the main experiment) or the positive control chemical (chlorpromazine, CPZ). Vehicle control treatments of 1% dimethyl sulphoxide (DMSO) in Hank's Balanced Salt Solution (HBSS) and blanks (media only) were also included on each plate. The cultures were treated for 1 hour at 37°C prior to irradiation. One set of plates was exposed to 5 J/cm^2 UV-A light and a second set of plates was protected from light for the same time period. After irradiation, the medium was aspirated from each well, cells washed with HBSS, and finally 0.15 mL medium was added to each well. The plates were then incubated for 20±2 hours at 37°C, 5% CO_2; 90% humidity. At the end of the incubation period, cytotoxicity was assessed by the Neutral Red Uptake assay. Irradiated vehicle controls showed a viability of at least 80% of the non-irradiated vehicle controls and the OD_{540} in the vehicle controls was >0.4 in both experiments. The IC_{50} values for the positive control were within the required range and the Photo Impact Factor (PIF) for the positive control (CPZ) was >6 in the main experiment. Therefore the validity and sensitivity of the assay was demonstrated. Treatment of cultures with Gibberellic Acid A4/A7 resulted in no IC_{50} values in either the absence and or in presence of UV-A light in either experiment. The PIF values were not calculated and were considered at 1.00 by default.

Under the experimental conditions of this study, the test item, Gibberellic Acid A4/A7, tested at up to 1000 µg/mL, was determined not to be phototoxic according to the classifications presented in the OECD Guideline 432.

I Materials and Methods

A. Materials:

1.	**Test Material**	Gibberellic Acid A4/A7
	Description	White powder
	Lot/Batch	1000048922
	Purity	90.16%
	CAS	468-44-0 (Gibberellin A4); 510-75-8 (Gibberellin A7)
	Stability	Confirmed stable for the duration of the study
	Solvent	1% dimethyl sulphoxide (DMSO) in Hank's Balanced Salt Solution (HBSS)
2.	**Controls**	
	Vehicle / Final Concentration	1% DMSO in HBSS.
	Positive (-UV-A)	Chlorpromazine (CPZ) in HBSS at 4.71, 10.13, 21.77, 46.8, 100.62, 216.33, 465.12, 1000 µg/mL.
	Positive (+UV-A)	CPZ in HBSS at 0.01, 0.03, 0.1, 0.32, 1, 3.16, 10, 31.6 µg/mL.
3.	**UV Irradiation**	The UV source was a UVACUBE 400 (SOL-500 lamp) fitted with a filter against UV-B emission (H1-filter).
4.	**Test Cells**	Mouse fibroblasts (Balb/c 3T3 clone A31), from the American Type Culture Collection, supplied by LGC Standards, UK were maintained at CiToxLab in culture medium at 37°C in an atmosphere of 5% CO_2 in air. The passage number of the cells did not exceed 100.
5.	**Culture medium**	Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% calf serum, 4 mM glutamine, 100 IU penicillin, 100 µg/mL streptomycin.
6.	**Concentrations**	-UV-A / +UV-A (preliminary test): 0, 0.32, 1, 3.17, 10.03, 31.69, 100.15, 316.46,

1000 µg/mL
-UV-A / +UV-A (main test): 0, 263.34, 318.64, 385.55, 466.51, 564.48, 683.02, 826.45, 1000 µg/mL

B. Test Performance

1. In-life dates

01 August 2017 to 10 August 2017.

2. Cell culture preparation

Aliquots (100 µL) of supplemented DMEM were dispensed into the peripheral wells of four 96-well plates, the remaining wells received 1 x 10^4 cells in 100 µL culture medium. Two plates were set up for the test material and two for the positive control. One column of six wells was used for each concentration of test material or positive control. Two columns of six wells were used for vehicle. Plates were incubated at 37°C, 5% CO_2; 90% humidity for approximately 24±2 hours until they formed a half-confluent monolayer.

3. Cell treatment

Culture medium was removed from appropriate wells of the 96-well plates; wells were washed with pre-warmed HBSS and then 100 µL of vehicle, test material or positive control solutions were added to the appropriate wells. HBSS was applied to the peripheral wells. All plates were incubated at 37°C, 5% CO_2; 90% humidity for 60±5 minutes. On completion of incubation, one plate for the test material and one plate containing the positive control was irradiated using the UV-A light source for 50 minutes at room temperature, to achieve a UV-A dose of 5 J/cm^2. Irradiation was conducted through the lids of the 6-well plates. The remaining plates were maintained at room temperature protected from light for the same time period. Following treatment, test solutions were removed from the wells, cells were washed at least twice with HBSS and 150 µL pre-warmed fresh DMEM was added to each well. The plates were then incubated at 37°C, 5% CO_2; 90% humidity for 18-22 hours.

4. Cytotoxicity (visual evaluation and Neutral Red Uptake)

At the end of the incubation period, cells were briefly examined microscopically for signs of cytotoxicity. Immediately following the visual assessment, the cells were washed with HBSS. This was removed, and 100 µL of neutral red solution (50 µg/mL in media) was added to each well. The plates were incubated at 37°C, 5% CO_2; 90% humidity for approximately 3 hours ± 6 minutes. Following the incubation, the neutral red solution was removed and the cells washed with HBSS. Desorption solution (150 µL) (ethanol:glacial acetic acid:distilled water, 50:1:49) was added. Plates were shaken to allow extraction of neutral red from the cells. Optical densities (ODs) of each well were read on a plate reader, at a wavelength of 540 nm, using the optical densities of the peripheral wells as blanks.

5. Analysis of results

Where possible, the concentration of the test material inducing a 50% inhibition of Neutral Red Uptake (IC50 value) was calculated for the test material and the positive control, using the software PHOTOTOX, version 2.0, as cited in OECD 432. Additionally, a Photo Impact Factor (PIF) and a Mean Photo Effect (MPE) values were generated.

PIF was calculated using the following formula: PIF = IC_{50}(Irr-)/IC_{50}(Irr+).
In the case where no IC_{50} values could be calculated for the test item or CPZ, the PIF value was not calculated and was considered as 1.00 by default.

In cases where an IC_{50} was calculated for the irradiated plate but not for the non-irradiated plate, an accurate PIF cannot be calculated. However, an approximate PIF is calculated and used to evaluate the phototoxic potential. In this case (test item or CPZ), the maximum tested concentration in the non-irradiated plate is used as the IC_{50}(Irr-) to calculate the approximate PIF. This PIF value is referred to as > PIF in the study report and as "C PIF value" in the table processed by the PHOTOTX software. In that case, the formula is the following > PIF = highest tested concentration (Irr-)/IC_{50}(Irr+).

MPE is based on comparison of the complete concentration response curves. This prediction model is useful in the data evaluation if no IC_{50} is achieved for the irradiated or non-irradiated plate.
It is defined as the weighted average across a representative set of photo effect values.

$$MPE = \frac{\sum_{i=1}^{n} w_i \, PE_{c_i}}{\sum_{i=1}^{n} w_i}$$

The Photo Effect (PE_c) at any Concentration (C) is defined as the product of the Response Effect (RE_c) and the Dose Effect (DE_c) *i.e.* $PE_c = RE_c \times DE_c$. The Response Effect (RE_c) is the difference between the responses observed in the absence and presence of light, *i.e.* $RE_c = R_c$ (Irr-) - R_c (Irr+). The dose-effect is given by:

$$DE_c = \left| \frac{C/C^* - 1}{C/C^* + 1} \right|$$

where C* represents the equivalence concentration, *i.e.* the concentration at which the Irr+ response equals the Irr- response at concentration C. If C* cannot be determined because the response values of the Irr+ curve are systematically higher or lower than RC (Irr-) the dose effect is set to one. The weighting factors W_i are given by the highest response value, *i.e.* W_i = MAX {Ri (Irr+), Ri (Irr-)}. The concentration grid Ci is chosen such that the same number of points falls into each of the concentration intervals defined by the concentration values used in the experiment. The calculation of MPE is restricted to the maximum concentration value at which at least one of the two curves still exhibits a response value of at least 10%. If this maximum concentration is higher than the highest concentration used in the Irr+ experiment the residual part of the Irr+ curve is set to the response value "0".

6. Statistics

Not warranted.

7. Evaluation criteria

For valid data, the test material is considered to be phototoxic if Photo Impact Factor (PIF) values >5 or Mean Photo Effect (MPE) values >0.15 were obtained. The test material is considered to be not phototoxic if PIF values of ≤2 or MPE values ≤0.1 were obtained. The results are considered to be probably phototoxic if PIF values of >2 and ≤5 or MPE values >0.1 and ≤0.15 were obtained.
Since an IC50 was calculated for each plate (irradiated and non-irradiated), only the PIF value is taken into consideration for the final evaluation of the phototoxicity potential.

II Results and Discussion

A. Solubility test

In the solubility test, the test item was found not soluble in HBSS when tested at 1 mg/mL. However, it was found soluble in DMSO at 100 mg/mL and even after a 100 fold dilution of this formulation in HBSS. Therefore, according to the rational for test item concentration to be used in the preliminary test, the highest

soluble concentration (1000 µg/mL in HBSS containing 1% DMSO) was selected for the treatment of the preliminary test.

B. Preliminary test

Following treatment with the test substance in the absence of UV-A, no change in cell morphology was visualised at any tested concentration, no decrease in Neutral Red (NR) uptake was observed at any tested concentrations and no IC50 was calculated. Following treatment with the test substance in the presence of UV-A, a change in cell morphology was noted at concentrations ≥100.15 µg/mL and a decrease in NR uptake was observed at the concentration of 1000 µg/mL where viability values of individual replicate values varied between 44.5 and 84.3%. However, the cell viability was > 50% therefore no IC_{50} was calculated. As a consequence, the PIF value was also not calculated and was considered at 1.00 by default.

The positive control results were not reported.

C. Main test

Following treatment with the test substance, a change in cell morphology was observed only in the irradiated plate at 1000 µg/mL and there were no decrease in viabilities (no decrease in NR uptake) at any tested concentrations in the irradiated or non-irradiated plates.

No IC50 values were calculated. As a consequence, the PIF value was also not calculated and was considered at 1.00 by default (refer to Table CA 5.2.7-1).

Table CA 5.2.7-1 IC_{50} and PIF values

Test material	IC_{50} –UV-A (µg/mL)	IC_{50} +UV-A (µg/mL)	PIF Value
Gibberellic Acid A4/A7	a	a	1.00
CPZ	30.127[b]	0.521[c]	60.539[d]

a: cell survival at the highest concentration tested was more than 50% and hence IC_{50} values could not be calculated

b: IC_{50} was within 7.0-90 µg/mL, therefore acceptable

c: IC_{50} was within 0.1-2.0 µg/mL, therefore acceptable

d: PIF >6, therefore acceptable

The positive control, CPZ, induced an acceptable positive response with IC_{50} values within the appropriate ranges and a PIF value of >6, thus demonstrating the sensitivity of the assay. In the vehicle controls, OD_{540} values were greater than 0.4. The irradiated vehicle control showed a viability of at least 80% of the non-irradiated vehicle control. The assay was therefore considered valid.

D. Deficiencies

None.

III Conclusion

Under the experimental conditions of this study, the test item, Gibberellic Acid A4/A7, tested at up to 1000 µg/mL, was determined not to be phototoxic according to the classifications presented in the OECD Guideline 432.

RMS comments and conclusion:

The study is acceptable. It followed OECD 432 (2004) and principles of GLP. Negative and positive controls meet the acceptance criteria listed in the OECD 432. The report also contained historical control data from 16 experiments performed from January 2015 to March 2017. The preliminary and main tests were performed with cells at passages 83 and 87, respectively. According to the test report, the sensitivity of the cell line to UVA irradiation is checked every 6 months , the last being performed in July 2017. According to the criteria of the OECD 432, GA4/7 is not predicted to be phototoxic.

According to data requirements (Regulation 283/2013) this study is required for active substances which absorb electromagnetic radiation in the range 290-700 nm and are liable to reach the eyes and light-exposed areas of skin. The UV/VIS spectrum of GA4/7 was determined (Comb 1997 (KCA 2.1_01)). Based on the light absorption data GA4/7 absorbs the most of UV radiation in the part of the UV-C spectra (wavelengths of 100-280 nm). UV-C rays are absorbed in the ozone layer and do not reach the surface of Earth. Therefore, it is not relevant for the human exposure. According to the GA4/7 absorption spectra, it absorbs only a very small proportion of light at wavelengths in UV-B range. However, the visible molar absorption coefficient of GA4/7 is below 10 $L \times mol^{-1} \times cm^{-1}$ in methanol at wavelength $\geq$ 286 nm. According to the data provided in the study (Comb 1997) it is shown, that the criteria laid down in the Commission Regulation (EU) No 283/2013 for the performance of the phototoxicity study are not met.

Thus, the study on phototoxicity is not deemed to be required.

B.6.3. SHORT-TERM TOXICITY

B.6.3.1. Oral 28-day study

a) Previous evaluation (2005-2011)

A 28-day short-term study for GA4/7 was not considered necessary, since a 90-day feeding study has been already available for the rat.

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

A 28-day oral toxicity study is not required; according to Regulation (EU) No 283/2013 the study is not required as an acceptable 90-day oral toxicity study is available.

RMS comments and conclusion:

The 28-day oral toxicity study is not considered necessary, as an acceptable 90-day oral toxicity study is available.

B.6.3.2. Oral 90- day study

a) Previous evaluation (2005-2011)

Data on the toxicity of GA4/7 to rats and dogs following repeated oral exposure were submitted during the EU review and are available in the EU DAR. The studies below were considered acceptable in the EFSA conclusion and are considered adequate for supporting renewal of GA4/7; no new repeated dose toxicity studies are submitted. EFSA (2012) concluded that the relevant oral 90-day NOAEL in the dog is 650 mg/kg bw/day based on mild toxicity observed at the highest dose level of 1000 mg/kg bw/day (liver and kidney effects, reduced food consumption and body weight gain). EFSA (2012) concluded that the relevant oral 90-day NOAEL in the rat is 500 mg/kg bw/day based on kidney effects and reduced food consumption and body weight gain. Effects

observed in the rat were only noted at the highest dose level (50000/25000 ppm, equivalent to approximately 1250-2500 mg/kg bw/day), which is in excess of the limit dose of 1000 mg/kg bw/day.

B.6.3.2.1. Sub-chronic (90 days) rat study

PREVIOUS EVALUATION	This study was evaluated in the DAR and has been considered by EFSA.
Data point addressed:	CA 5.3.2/01 (B.6.3.1.2 DAR)
Author(s) (year):	[redacted] (1990)
Title:	13-Week Dietary Toxicity Study with Gibberellins A4/A7 in Rats
Laboratory report / project number:	[redacted]
Testing facility:	[redacted]
Published:	No
Test guideline used:	US EPA FIFRA Guideline 82-1, comparable to OECD 408
Deviations:	The study was equivalent to the earlier version of OECD 408 therefore some investigations were not performed (see below) however it is still considered valid and acceptable for characterising the sub-chronic toxicity of the substance
GLP:	Yes
EU Agreed Endpoint:	Relevant oral NOAEL: 90-day rat, 500 mg/kg bw/day

Executive summary

Gibberellins GA4/7 (85.5% purity, batch 21-018-CD) was administered for 90 days to groups of Crl:CD®BR rats in the diet at concentrations of 0, 1000, 10000 and 50000 ppm. The highest dose level was reduced from 50000 to 25000 ppm from Day 15 of treatment after adverse effects were apparent. Ten rats of each sex were retained from the control and high level groups for a 4 week recovery period after completion of 13 weeks of treatment. There were no treatment-related effects among rats treated at up to 10000 ppm (500 mg/kg bw/day). Findings in the high level group included reduced bodyweight gain, decreased food consumption, a single mortality (with clinical signs or reaction to treatment (hunched posture, bloody crust around nose and thin, rough haircoat) generally associated with administration of the 50000 ppm diet. Macroscopic changes in the high dose group kidneys were reflected histologically by tubulo-interstitial nephritis and nephron loss and possible liver changes were noted as hepatocellular vacuolation and degeneration. The NOAEL was 10000 ppm (500 mg/kg bw/day).

I Materials and Methods

A. Materials:

1. Test Material Gibberellins GA4/7

Description White powder

Lot/Batch 21-018-CD

Purity 85.5%

Stability Assumed to be stable for the duration of the study

2. Vehicle Diet

3. Test animals Rats

Species	Crl:CD BR albino
Age	43 days old at start of treatment
Weight	136.5 to 243.6 g males and females at start of treatment
Source	[redacted]
Acclimation period	15 days
Diet	Standard laboratory rodent diet (Purina Certified Rodent Chow #5002) *ad libitum*
Water	Tap water *ad libitum*
Housing	Animals were individually housed in suspended screen-bottomed stainless steel cages

4. Environmental conditions

Temperature	16-25.6°C
Humidity	24-69%
Air changes	Not stated
Photoperiod	12 hour light/dark cycle

B. Study Design and Methods

1. In life dates: 20 June to 18 October 1989

2. Animal assignment and treatment

Twenty or ten rats of each sex were allocated to study groups dosed for 13 weeks at dose levels of 0, 1000, 10000 or 50000 ppm. The time-weighted achieved dose levels were not calculated from analysis of achieved concentration, body weight and food consumption data but approximate values have been estimated using the assumptions set out by Lehman, A.J. (1954)[1] for approximate relation of part/million in the diet to mg/kg body weight/day. Assuming average weight of rat to be 400g and average food consumption of 20 g/rat/day, the achieved dose levels were 0, 50, 500 and 1250 mg/kg bw/day. Weighed test material was mixed by direct dilution with diet to produce each test concentration. Fresh test diets were prepared weekly and stored refrigerated prior to use. Each weekly prepared diet was analysed for achieved concentration, stability and homogeneity. All animals were observed at least twice daily for mortality, moribundity and evidence of clinical signs of toxicity throughout the study. In addition a detailed weekly physical examination of all animals was included. Body weights were measured prior to the start of treatment, on Day 1 (the day of dosing) and then at weekly intervals for the remainder of the observation period and at termination, after 13 weeks of treatment or at the end of the treatment free period. Diet consumption was measured at weekly intervals throughout the treatment and recovery phases of the study. The eyes of all rats were examined prior to start of treatment and again during Week 13 and, for the recovery group animals, at Week 17. Blood samples were obtained during Week 13 from the orbital sinus of all animals. In addition, samples were obtained from the untreated rats of group 5 during week 1 and from the recovery animals during Week 17. Standard assays were used to analyse the haematology and clinical chemistry parameters. All animals (including single decedent) were subject to gross pathological examination during necropsy that involved examination of external surfaces, all orifices and nasal, thoracic abdominal, pelvic and cranial cavities (external brain surface and cut surfaces of the spinal cord were also examined). The kidneys, liver, brain, adrenals, ovaries and testes were excised and weighed. Gross lesions and a full EC compliant list of tissues were examined histologically under light microscopy for the control and high dose groups, macroscopic lesions, lungs, liver and kidneys from animals of the low and intermediate groups were also examined. Livers and kidneys were examined from the recovery group

[1] Lehman, A.J. (1954) Association of Food and Drug Officials Quarterly Bulletin, 18: 66. The values presented in the table are average figures, derived from numerous sources

animals.

3. Statistics Standard parametric and non-parametric tests were used for comparison of group means

II Results and Discussion

A. Clinical signs and mortality

One male from the high level group (50,000 ppm / *approximately* 2500 mg/kg bw/day) died during Week 3. Ante-mortem signs for this animal included a hunched posture, a rough, thin pelage and a bloody crust around the nose. These signs were evident in other high level males treated at 50,000 ppm and did appear to be a reaction to dosing with gibberellins GA4/7. However, the signs were not evident after the dose was reduced to 25,000 ppm (*approximately* 1250 mg/kg bw/day). Necropsy of the decedent revealed no macroscopic abnormalities. Histopathological examination revealed renal changes including multifocal areas of papillary necrosis, cortical scarring/fibrosis and tubular dilatation. There were no unscheduled deaths following the high dose reduction.

Clinical signs of reaction to treatment were limited to the high level group, particularly associated with administration of 50,000 ppm as detailed above. Other clinical changes were limited to urine staining or urine stained tails for males and females of the high level group that were resolved during the recovery period.

B. Body weight

The body weight gains recorded for the high level group during Weeks 1 and 2 (the period of 50,000 ppm administration) were significantly lower than controls but the gains for Week 3 for this group were significantly higher than controls after changing to the lower level (25,000 ppm). Other than sporadic fluctuations in weight, the weight gains for the high level group were similar to or exceeded control gains for the period Week 3 to 13. Cumulative weight gains were lower for the intermediate level females in Week 4. Cumulative gains for the high level group were lower than controls throughout the study reflecting the weight losses sustained in weeks 1 and 2.

C. Food consumption

Food consumption by males treated at 50,000 ppm was significantly reduced (Week 1 to 3) and for the high level females in weeks 1, 2, 4 and 5. The reduction in the high dose concentration was reflected in food consumption values that were similar to controls for the high level group in the remainder of the study. There were no apparent treatment effects on food consumption for the low or intermediate groups.

D. Blood analysis

Haemoglobin and haematocrit values were lower than controls for the high level group. There were no other notable changes in haematology parameters. Total protein, albumin and calcium levels were lower in the high level females than the controls. Globulin values were higher than controls for the high level females. Increases in total bilirubin, cholesterol and alkaline phosphatase were apparent for both males and females of the high level group.

E. Ophthalmoscopy

There were no treatment related changes apparent at either week 13 or 17.

F. Sacrifice and pathology

Lower terminal bodyweights for the high level group, as a consequence of effects on weight gain of the 50,000 ppm administration in Weeks 1 and 2, resulted in some changes in organ weight or relative organ weight but these did not appear to be directly related to treatment with gibberellins GA4A7 at 25,000 ppm. There were no treatment-related effects on absolute or relative organ weights in either sex at the low or intermediate dose levels. The high dose group had a high incidence of kidney changes including a diffusely rough surface or depressed foci/areas on the cortical surface. There were no macroscopic changes considered to be treatment-related among rats dosed at 10,000 or 1,000 ppm. Histopathological findings at week 14 were limited to the high dose group. Findings comprised a high incidence of chronic or chronic active tubulointerstitial nephritis with tubular dilation and focal nephron loss. The high dose group also had a higher incidence of hepatocellular vacuolation with associated hepatocellular

degeneration. The effects did not extend to the low or intermediate dose group. Histopathological investigations after the recovery period, week 18, were limited to examination of the liver and kidney. At this point there was no evidence of hepatocellular degeneration and only single incidences of hepatocellular vacuolation in two different sex/dose groups. High dose group animals showed renal changes that included chronic or chronic-active inflammation, cortical fibrosis/scarring and tubular dilation. The findings were more severe and more prevalent among males than females but less severe in both sexes than similar changes noted at week 14. Based on the in-life and pathological data the intermediate level 10,000 ppm (approximately 500 mg/kg bw/day) was considered to be the NOAEL in this study. The low dose level, 1000 ppm, (approximately 50 mg/kg bw/day) was an NOEL.

Table B.6.3.2/01-1 Selected haematology findings

	Males				**Females**			
Diet concentration (ppm)	**0**	**1000**	**10000**	**25000**	**0**	**1000**	**10000**	**25000**
Dose level (mg/kg bw/day)	**0**	**50**	**500**	**1250**	**0**	**50**	**500**	**1250**
Number of animals/group	**20**	**10**	**10**	**19**	**20**	**10**	**10**	**20**
Haematology (mean values)								
Haemoglobin (Week 13)	14.5	15.3*	14.7	14.0*	14.1	14.3	14.4	13.3
Haematocrit (Week 13)	53.2	54.7	53.2	50.9*	51.7	51.5	51.3	48.8
Haemoglobin (Week 17)	13.6	--	--	13.8	13.8	--	--	13.2
Haematocrit (Week 17)	50.1	--	--	51.2	50.9	--	--	49.1

* Statistically significant difference from controls. $p<0.05$, using Student's t-test or Dunnett's test.

Table B.6.3.2/01-2 Selected clinical chemistry, organ weight and pathology findings

	Males				**Females**			
Diet concentration (ppm)	**0**	**1000**	**10000**	**25000**	**0**	**1000**	**10000**	**25000**
Dose level (mg/kg bw/day)	**0**	**50**	**500**	**1250**	**0**	**50**	**500**	**1250**
Number of animals/group	**20**	**10**	**10**	**19**	**20**	**10**	**10**	**20**
Blood chemistry (mean values)								
Total protein (Week 14)	7.0	6.9	7.0	7.0	7.3	7.2	7.0	6.8*
Albumin (Week 14)	4.4	4.4	4.4	4.4	5.2	5.0	4.7*	4.4*
Globulin (Week 14)	2.5	2.5	2.6	2.6	2.2	2.2	2.3	2.4*
Calcium (Week 14)	10.1	10.0	10.0	10.2	10.4	10.3	10.1	10.0*
Total bilirubin (Week 14)	0.2	0.2	0.2	0.2*	0.2	0.2	0.2	0.2*
Cholesterol (Week 14)	59	56	68	90*	71	71	73	85*
Alkaline phosphatase (Week 14)	96	109	95	124*	61	63	61	87*
Total protein (Week 18)	6.9	--	--	6.9	7.5	--	--	7.2*
Albumin (Week 18)	4.4	--	--	4.5	5.4	--	--	4.9*
Globulin (Week 18)	2.5	--	--	2.4	2.1	--	--	2.3
Calcium (Week 18)	9.9	--	--	9.9	10.3	--	--	10.1
Total bilirubin (Week 18)	0.2	--	--	0.2	0.3	--	--	0.2

Cholesterol (Week 18)	62	--	--	61	76	--	--	74
Alkaline phosphatase (Week 18)	84	--	--	105	62	--	--	68

* Statistically significant difference from controls. $p<0.05$, using Student's t-test or Dunnett's test.

Table B.6.3.2/01-3 Selected organ weight and pathology (non-recovery groups)

	Males				**Females**			
Diet concentration (ppm)	**0**	**1000**	**10000**	**25000**	**0**	**1000**	**10000**	**25000**
Dose level (mg/kg bw/day)	**0**	**50**	**500**	**1250**	**0**	**50**	**500**	**1250**
Number of animals/group	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Organ weight (g. absolute week 14)								
Left kidney	1.817	1.859	1.781	1.730	0.933	0.933	0.950	0.839
Right kidney	1.843	1.850	1.724	1.710	0.930	1.020	0.929	0.843
Liver	15.65	15.58	15.74	14.45	7.23	6.86	7.25	6.99
Organ weight (% relative to body weight week 14)								
Terminal bodyweight	556.1	542.2	525.2	456.8	254.7	255.4	260.8	228.9
Left kidney	0.328	0.344	0.340	0.381 *	0.369	0.368	0.366	0.369
Right kidney	0.332	0.342	0.328	0.377 *	0.368	0.403	0.359	0.370
Liver	2.824	2.870	2.991	3.156	2.850	2.690	2.794	3.059
Organ weight (ratio relative to brain weight week 14)								
Terminal brain weight	2.156	2.143	2.100	2.101	1.975	1.926	1.949	1.981
Left kidney	0.843	0.867	0.848	0.825	0.473	0.484	0.488	0.424
Right kidney	0.855	0.862	0.820	0.815	0.471	0.528	0.477	0.427
Liver	7.263	7.265	7.483	6.871	3.657	3.568	3.720	3.536
Histopathology incidence (Week 14)								
Liver								
mononuclear cell infiltrates	7	7	8	8	5	6	7	10
coagulative necrosis	1	0	0	0	0	0	0	0
centrilobular hepatocyte vacuolation	0	0	1	8	0	0	1	2
centrilobular hepatocellular degeneration	0	0	0	3	0	0	0	0

Kidney								
pelvic dilatation	1	2	0	2	0	2	0	1
tubular mineralisation	1	0	0	0	1	3	4	3
chronic/chronic active inflammation ##	0	0	0	8	0	0	0	4
proteinaceous casts (in dilated tubules)	0	0	3	2	0	0	0	1
cortical fibrosis/scarring	0	0	0	9	0	1	0	7
cyst(s)	0	0	0	5	0	0	0	3
tubular atrophy	0	0	2	2	0	0	0	7
suppurative pyelonephritis	0	0	0	0	0	1	0	0
tubular dilation	0	0	0	10	0	0	0	2

* Statistically significant difference from controls. $p<0.05$, using Student's t-test or Dunnett's test.

characterised by presence of mononuclear cell infiltrates, fibrosis, chronic tubular atrophy (chronic) and/or polymorphonuclear cells with some necrotic debris (active). Tubulo-interstitial nephritis and glomerulitis.

Table B.6.3.2/01-4 Selected organ weight and pathology findings at termination (4 week recovery groups)

	Males		**Females**	
Diet concentration (ppm)	**0**	**25000**	**0**	**25000**
Dose level (mg/kg bw/day)	**0**	**1250**	**0**	**1250**
Number of animals/group	**10**	**9**	**10**	**10**
Organ weight (g. absolute week 18)				
Left kidney	1.955	1.849	1.001	0.892*
Right kidney	1.986	1.899	1.002	0.894*
Liver	16.57	14.34*	6.99	6.89
Organ weight (% relative to body weight week 18)				
Terminal bodyweight	571.9	518.7*	276.1	246.5*
Left kidney	0.342	0.358	0.363	0.362
Right kidney	0.348	0.368	0.364	0.363
Liver	2.889	2.768	2.535	2.799*
Organ weight (ratio relative to brain weight week 18)				
Terminal brain weight	2.1414	2.1805	1.9816	1.9912
Left kidney	0.914	0.848	0.506	0.448*
Right kidney	0.928	0.871	0.506	0.449*
Liver	7.741	6.584*	3.532	3.466
Histopathology incidence (Week 18)				
Liver				
mononuclear cell infiltrates	9	6	3	5
coagulative necrosis	0	0	0	1
centrilobular hepatocyte vacuolation	1	0	0	1
inflammation	0	0	0	1

Kidney				
pelvic dilatation	1	0	2	0
tubular mineralisation	0	1	4	2
chronic/chronic active inflammation ##	0	7	0	5
proteinaceous casts (in dilated tubules)	3	3	1	2
cortical fibrosis/scarring	1	8	0	10
cyst(s)	1	3	0	0
tubular atrophy	7	2	0	0
tubular dilation	0	9	0	2

* Statistically significant difference from controls. $p<0.05$, using Student's t-test or Dunnett's test.

characterised by presence of mononuclear cell infiltrates, fibrosis, chronic tubular atrophy (chronic) and/or polymorphonuclear cells with some necrotic debris (active). Tubulo-interstitial nephritis and glomerulitis.

Conclusion

The 90-day rat oral NOAEL was 500 mg/kg bw/day. Classification according to Regulation (EC) No 1272/2008 is not required. The endpoint was agreed in the EFSA Conclusion and is still considered valid.

RMS comments and conclusion:

The study was done according to a guidance comparable to an old version of OECD 408 (1981) and follows principles of GLP. The latest version of OECD 408 (2018) was updated to add endocrine-sensitive endpoints. The observed deviation of the study compared to the latest version of OECD 408 (2018) are: no conduction of sensory activity, grip strength and motor activity assessments, omission of urinalysis (optionally), they did not weight: epididymis, prostate + seminal vesicles with coagulating glands, uterus, thymus, spleen, heart, pituitary gland and thyroid gland; the histopathology was not performed for: male mammary glands, coagulating glands, and vaginal smear; payers patches were not specifically mentioned; no analyses of serum/plasma hormones (T4, T3, TSH, FSH, LH, oestradiol, testosterone) and HDL, LDL and no sperm measures (cauda epididymis sperm reserves, sperm motility, sperm morphology) according Annex B of OECD 408 were done. Evaluation of possible endocrine disrupting properties is discussed in B6.8.3. The current study is considered to have some limitations, but to be acceptable for the analysed endpoints.

Analysed information relevant for the risk assessment were reported in tabular form. A male dosed at the highest dose, 50,000 ppm, died during the study. Due to excessive toxicity the dose was reduced to 25,000 ppm on day 15. Afterward, only urine staining was observed at the highest tested dose. Body weight gains and food consumption data are presented in Table B.6.3.2/01-4 to 6.3.2/01-7. Body weight gains were lower for males and females dosed at the highest dose (week 1-2 with 50,000 ppm, afterwards with 25,000 ppm). The body weight gains were around or above the controls from week 3 onwards.

Table B.6.3.2/01-4 Body weight, weight gain and cumulative weight gain for male rats dosed over 13 weeks with GIBBERELLINS GA4/GA7 (DAR, 2011)

Dose (ppm)	**Males**											
	0			**1000**			**10,000**			**50,000 25000**		
	A	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**
Week												
0	228.0			225.3			225.0			219.6		
1	288.6	60.6	60.6	288.4	63.1	63.1	284.4	59.4	59.4	221.5	1.8	1.8
2	336.6	48.0	108.6	338.7	50.3	113.4	332.9	48.4	107.8	229.0	7.6	9.4
3	381.8	45.2	153.8	380.3	41.6	155.0	374.2	41.3	149.1	292.2	58.8	71.8
4	411.6	29.8	183.6	409.5	29.2	184.2	399.7	25.6	174.7	329.8	37.6	109.4

5	443.2	31.6	215.2	435.0	25.5	209.7	426.5	26.8	201.5	365.2	35.4	144.8
6	463.1	19.9	235.1	457.8	22.8	232.5	451.5	25.1	226.5	386.7	21.5	166.3
7	491.3	28.1	263.3	476.5	18.7	251.2	472.2	20.6	247.1	410.7	24.0	190.3
8	515.0	23.7	287.0	501.5	25.0	276.2	490.7	18.5	265.7	426.1	15.5	205.8
9	538.9	23.9	310.9	527.7	26.2	302.4	511.5	20.8	286.5	449.8	23.6	229.4
10	548.7	9.8	320.7	537.0	9.3	311.6	523.3	11.8	298.3	459.8	10.0	239.4
11	564.6	15.8	336.6	552.4	15.4	327.1	537.9	14.6	312.9	472.8	13.0	252.4
12	577.5	12.9	349.5	561.1	8.6	335.7	551.0	13.0	325.9	487.0	14.3	266.7
13	588.7	11.2	360.7	574.5	13.5	349.2	562.3	11.3	337.3	496.4	9.4	276.1
14	571.0	-14.4	344.0	--	--	--	--	--	--	502.1	-4.3	283.9
15	589.7	18.6	362.6	--	--	--	--	--	--	527.1	24.9	308.8
16	598.5	8.8	371.4	--	--	--	--	--	--	540.5	13.4	322.2
17	615.8	17.4	388.8	--	--	--	--	--	--	560.6	20.2	342.4

A Actual body weight (g), B Weekly bodyweight gain (g), C cumulative weekly gain (g), Grey shaded values were significantly different from controls, $p<0.05$.

Table B.6.3.2/01-6 Body weight, weight gain and cumulative weight gain for female rats dosed over 13 weeks with GIBBERELLINS GA4/GA7 (DAR, 2011)

Dose (ppm)	**Females**											
	0			**1000**			**10,000**			**50,000 25,000**		
	A	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**
Week												
0	146.0			146.8			151.6			149.4		
1	168.4	22.3	22.3	169.6	22.8	22.8	173.4	21.8	21.8	151.4	2.0	2.0
2	188.9	20.6	42.9	188.9	19.2	42.1	191.4	18.0	39.7	165.1	13.7	15.7
3	205.3	16.4	59.3	205.4	16.5	58.6	209.0	17.7	57.4	185.9	20.8	36.5
4	216.4	11.1	70.3	214.1	8.7	67.3	210.9	1.9	59.3	195.6	9.7	46.2
5	229.6	13.3	83.6	225.4	11.3	78.6	230.8	19.8	79.2	205.9	10.3	56.5
6	238.7	9.1	92.7	236.8	11.4	90.0	241.0	10.2	89.4	213.6	7.7	64.2
7	247.9	9.2	101.9	244.1	7.3	97.3	249.6	8.6	98.0	224.3	10.7	74.9
8	250.1	2.2	104.1	245.3	1.2	98.5	251.4	1.7	99.7	222.5	-1.8	73.1
9	262.6	12.5	116.6	257.9	12.7	111.1	262.3	10.9	110.7	234.2	11.6	84.8
10	266.2	3.6	120.2	261.2	3.2	114.4	267.0	4.7	115.4	236.5	2.3	87.1
11	272.9	6.7	126.9	267.3	6.2	120.5	273.5	6.5	121.8	243.8	7.3	94.4
12	278.6	5.7	132.6	272.5	5.1	125.6	280.0	6.5	128.4	247.8	4.0	98.4
13	282.1	3.5	136.1	278.0	5.5	131.2	283.8	3.8	132.1	252.1	4.3	102.7
14	279.6	-6.0	132.5	--	--	--	--	--	--	249.7	-2.0	97.7
15	288.2	8.6	141.0	--	--	--	--	--	--	257.8	8.0	105.7
16	294.2	6.0	147.0	--	--	--	--	--	--	263.9	6.2	111.9
17	303.5	9.3	156.4	--	--	--	--	--	--	275.0	11.1	123.0

A Actual body weight, B Weekly bodyweight gain, C cumulative weekly gain, Grey shaded values were significantly different from controls, $p<0.05$.

Table B.6.3.2/01-7 Food consumption for rats dosed over 4 weeks with GIBBERELLINS GA4/GA7 (DAR, 2011)

	Males				**Females**			
Dose (ppm)	**0**	**1000**	**10000**	**25000**	**0**	**1000**	**10000**	**25000**
(mg/kg bw)	**0**	**50**	**500**	**1250**	**0**	**50**	**500**	**1250**
Week								
1	185.5	184.3	185.7	129.2*	120.5	125.8	122.0	87.4*

2	187.6	193.5	197.1	150.4*	129.2	128.3	123.2	109.7*
3	189.8	190.6	198.5	178.8*	126.7	125.4	126.6	108.3
4	195.3	199.4	202.1	199.5	133.8	131.6	133.9	119.9*
5	193.6	196.2	199.9	200.2	134.5	137.3	129.4	122.0*
6	193.3	188.0	201.0	197.6	133.8	136.8	133.8	124.5
7	196.8	189.5	201.4	191.2	127.4	131.8	128.5	120.3
8	194.3	198.7	197.5	183.3	117.1	117.3	119.5	115.3
9	202.3	200.9	203.1	197.2	129.4	142.4	122.2	123.1
10	196.6	192.8	200.0	187.5	124.5	135.5	130.1	118.5
11	191.6	197.8	200.1	185.9	128.0	125.8	126.7	119.0
12	193.9	188.7	202.8	189.1	124.3	130.6	130.5	120.8
13	188.2	191.9	191.4	182.2	118.3	121.6	127.1	115.0
14	156.4	--	--	160.3	95.0	--	--	101.1
15	192.6	--	--	185.9	129.8	--	--	122.8
16	187.6	--	--	183.7	128.0	--	--	128.7
17	184.0	--	--	177.1	118.5	--	--	114.0

* Statistically significant difference from controls. $p<0.05$, using Student's t-test or Dunnett's test.

Statistically significant treatment related effect when compared to the control were found at the highest dose tested: ↓ Hb level (♂:3%, ♀: 6%), ↓ Ht level, (♂:9%, ♀: 6%), ↓ total protein (♀: 6%), ↓albumin (♀:1,5%) levels and ↑ globulin levels (♀: 9%), ↑cholesterol (♂: 52%, ♀: 20%), ↑ALP (♂: 30%, ♀: 43%), ↑bilirubin. The results point on possible adverse effects on liver and kidney and coincide with the pathology findings at that dose, which are more pronounced for males. The treatment related effects in the kidney were: chronic inflammation (♂: 8/10, ♀: 4/10), cortical fibrosis (♂: 9/10, ♀: 7/10), tubular dilation (♂: 10/10, ♀: 2/10), ↑relative kidney weight (♂:15%), ↓ absolute kidney weight (♀: 10%). Liver effects were limited to hepatocyte vacuolation (♂: 8/10, ♀: 2/10) and degeneration (♂:3/10).

After the recovery period, all haematological and blood clinical chemistry parameters for both sexes, except total protein and albumin levels in females were comparable to the controls. The pathological findings found at the end of week 13, were not reversible for kidney: chronic inflammation (♂: 7/9, ♀: 5/10), cortical fibrosis (♂: 8/9, ♀: 10/10), tubular dilation (♂: 9/9, ♀: 2/10).

As indicated by several findings the target organs of GA4/7 in the respective study are liver and kidneys. Based on the study results a NOAEL is set at 10000 pm (500 mg/kg bw/day). The same NOAEL was agreed at the Pesticide Peer Review Meeting 88 in September 2011. The estimation of the equivalent dose in mg/kg bw/day was done using a standard factor of 0.05, listed in the Guidance document for WHO monographers and reviewers (2015).

B.6.3.2.2. Sub-chronic (90 days) dog study

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 5.3.2/02 (B.6.3.1.2 DAR)
Author(s) (year):	[redacted] (2001)
Title:	ABG-3192 13-Week Oral (Capsule) Toxicity Study in Dogs
Laboratory report / project number:	[redacted]

Testing facility:	[redacted]
Published:	No
Test guideline used:	OECD 409 (1998), US EPA FIFRA Guideline 870.3150
Deviations:	None
GLP:	Yes
EU Agreed Endpoint:	Relevant oral NOAEL: 90-day dog, 650 mg/kg bw/day

Executive summary

Gibberellins GA4/7 (ABG-3192, 90% purity (A4 73.1%), batch 57-601-CD) was administered orally (via capsule) for 13 weeks to groups of 4 male and 4 female Beagle dogs at concentrations of 0, 330, 720 and 1100 mg/kg bw/day (equivalent to 0, 300, 650 and 1000 mg active substance/kg bw/d). There were no unscheduled deaths during the study. Treatment with 1100 mg/kg bw/day resulted in a slight reduction in food consumption and a consequent slight reduction in bodyweight gain. Liver and kidney weights were slightly elevated but in the absence of any corroborative evidence from clinical or microscopic pathology, these appeared to be minor adaptive responses with no adverse effect on the animals. Thymic atrophy and vacuolation of the zona fasciculata of the adrenal cortex and mild adrenal cortical atrophy were considered to be secondary effects indicative of stress and not directly related to administration of the test substance. In conclusion, there were no treatment-related effects among dogs dosed at 720 mg/kg bw/day and the findings in the high dose group were largely adaptive. The NOAEL established in this study was 720 mg ABG-3192/kg bw/d, equivalent to 650 mg active substance/kg bw/d.

I Materials and Methods

A. Materials:

1. Test Material ABG-3192, comprising a mixture of tetracyclic diterpenes Gibberellins A4 and A7

Description: Neutral white powder

Lot/Batch: 57-601-CD

Purity: 90% w/w (sum of GA4 and GA7); A4 comprised 73.1% w/w of mixture

Stability: Assumed to be stable for the duration of the study

2. Vehicle None

3. Test animals Dogs

Species: Beagle

Age: Approximately 4 months on arrival and 5 months at time of initial dose administration

Weight: 6.4 to 9.6 kg at time of arrival
8.0 to 10.8 kg males and 6.6 to 9.9 kg females at start of treatment

Source: [redacted]

Acclimation period: 4 weeks

Diet: SDS Dog Diet A [E] SQC. 400 g/day weighed ration supplied after each daily dosing occasion

Water: Tap water *ad libitum*

Housing: Animals were pair housed (2 dogs of same sex, same dose group) in double pens that enabled each pair to be separated for dosing, clinical observations and feeding

4. Environmental conditions

Temperature	15-23°C
Humidity	37-73%
Air changes	15/hour
Photoperiod	12 hour light/dark cycle

B. Study Design and Methods

1. In life dates: 3 January to 28 June 2001

2. Animal assignment and treatment

Four dogs of each sex were allocated to study groups dosed for 13 weeks at dose levels of 0, 330, 720 or 1100 mg ABG-3192/kg bw/d (equivalent to 0, 300, 650 or 1000 mg active substance/kg bw/d). The test material was weighed into a clear gelatin capsule for each daily dose. Weights were adjusted weekly to take account of most recent body weight recording. A fresh set of seven capsules was prepared up to one week in advance. Duplicate capsules were prepared for one male and one female in Weeks 6, 9 and 13. The duplicates were analysed for stability and accuracy of preparation. All animals were observed at least four times daily for evidence of clinical signs of toxicity or ill-health throughout the study. In addition, pens were inspected for evidence of ill health (vomitus, blood or abnormal faecal matter) daily and when dogs were removed from pens for exercise or pen cleaning, activity, gait and posture were checked. Body weights were measured at weekly intervals from two weeks prior to dosing up to termination after 13 weeks of treatment. Diet consumption was measured at weekly intervals from two weeks prior to dosing and throughout the treatment phases of the study. The eyes of all dogs were examined by indirect ophthalmoscope, prior to start of dosing and again during Week 6 and 13, following administration of a mydriatic agent. Blood samples were obtained pre-dosing and during Weeks 6 and 13 from the jugular vein of fasted animals. Standard assays were used to analyse the following parameters:-

haematology-erythrocyte count (RBC); platelet count; haematocrit (Hct); haemoglobin (Hb); reticulocytes (Reti); red blood cell indices (mean corpuscular volume [MCV], haemoglobin [MCH] and haemoglobin concentration [MCHC]); leukocyte count (WBC) and leukocyte differential count. Coagulation tests for prothrombin time (PT) and activated partial thromboplastin time (APTT).

clinical chemistry –glucose; albumin; globulin; total protein; blood urea nitrogen (BUN); bilirubin; alanine aminotransferase activity (ALT); aspartate aminotransferase activity (AST); alkaline phosphatase (AP); creatine phosphokinase (CPK); lactate dehydrogenase (LDH); sodium; potassium; chloride; calcium; inorganic phosphorus; creatinine; cholesterol; triglycerides and gamma glutamyl transferase (gGT).

Urinalysis was performed on animals held overnight, without food or water in metabolism cages, pre-trail and during Weeks 6 and 13. Faecal and urine samples were collected. Standard assays were used to analyse the following parameters:-

urinalysis-volume; specific gravity; pH; protein, glucose, ketones, urobilinogen, bilirubin, blood pigments and spun deposits were microscopically examined.

faeces-faecal occult blood

All animals were subject to gross pathological examination during necropsy that involved examination of external surfaces, all orifices and thoracic, abdominal and cranial cavities. The adrenals, brain, heart, liver, kidneys, lungs, ovaries, pancreas, pituitary gland, prostate gland, spleen, testes, thymus, thyroid and parathyroid, and uterus with cervix were excised and weighed. Gross lesions and a full EC compliant list of tissues from all dogs were examined histologically.

3. Statistics Standard parametric and non-parametric tests were used for comparison of group means

II Results and Discussion

A. Clinical signs and mortality

There were no unscheduled deaths during the study. Vomiting/regurgitation and voiding loose/liquid faeces were apparent in all groups at a low incidence (<21 or <29% respectively) and were not considered to be treatment related. One high dose male showed signs consistent with idiopathic febrile necrotising arteritis (periarteritis or "stiff beagle syndrome"). Signs developed on Day 64 and persisted intermittently during a course of steroidal treatment prescribed by the veterinarian. Other clinical signs were sporadic and showed no apparent dose or treatment-relationship

B. Body weight

The body weight gains recorded for the high dose group were slightly lower than controls although the effect did not achieve statistical significance. The effects did not extend to animals of the intermediate or low dose groups.

C. Food consumption

Food consumption by the high dose dogs was slightly but not significantly lower than controls. The slight reduction in consumption apparent for females dosed at 330 or 720 mg/kg bw/day was attributed to normal background variation in diet consumption rather than being a treatment effect. This was consistent with the body weight findings that showed no effect in the lower dose groups and only marginal effects in the high dose group.

D. Blood analysis

None of the haematological parameters investigated showed any effect of treatment with ABG-3129 (gibberellins GA4A7). Disruption in white blood cell count and increases in neutrophils, monocytes and large unclassified cells for one high dose male were attributed to the condition of periarteritis evident for this animal. Statistically significant increases in mean cell haemoglobin concentration were noted at week 13 for females dosed at 330 or 1100 mg/kg bw/day. However, the increase was slight in comparison with control and pre-dose values and showed no consistent time or dose related change and was not considered attributable to an effect of ABG-3192 administration. Investigations during week 6 revealed a number of parameters with lower values in the high dose group than in the corresponding control group. Toxicologically significant events are normally manifest by increases in these parameters. There was no consistent pattern of response and values were either similar to controls or pre-dose values or were within the normal background range for parameters with a high degree of variability. Evaluation of results from samples collected at termination also gave no indications of any treatment-related response. In the absence of any corroborative histopathological changes, none of the clinical chemistry findings were considered attributable to treatment with ABG-3129.

E. Urinalysis and faecal analysis

None of the urinalysis parameters were affected by treatment with ABG-3129. All animals were negative for the presence of occult blood in faeces.

F. Ophthalmoscopy

Several minor ocular changes were observed prior to starting dosing among dogs allocated to control and treated groups. Similar effects were also noted among all groups during ophthalmic examinations in Week 6 and 13. However, there were no changes that showed any dose–relationship or any increase in incidence to indicate a treatment related effect.

G. Sacrifice and pathology

Liver and kidney weights for dogs dosed at 720 or 1100 mg/kg bw/day were increased in comparison with controls but there were no histopathological findings to explain or corroborate the weight changes. The thymus weights for 3/4 of the high dose males were small. There were no treatment-related effects on absolute or relative organ weights in either sex at the low or intermediate dose levels. There were no gross abnormalities indicative of any effect of treatment with ABG-3129.

One high dose male, treated in-life for periarteritis, showed moderate diffuse adrenal cortex vacuolation and cortical atrophy, lymphoid atrophy of mandibular and mesenteric lymph nodes, thymic atrophy and marked diffuse hepatocyte vacuolation. The observed changes were considered to be consistent with the steroidal treatment received and not attributable to dosing with ABG-3129 Other histological changes of note were limited to two high dose males with mild/moderate thymic atrophy and one of these also showed vacuolation of the *zona fasciculata* of the adrenal cortex and mild adrenal cortical atrophy. No histopathological findings considered attributable to treatment with ABG-3219 were observed among the females at any dose level or among males dosed at 720 or 330 mg/kg bw/day.

Table B.6.3.2/02-1 Selected organ weight and pathology findings at termination

	Males				**Females**			
Dose level (mg/kg bw/day)	**0**	**330**	**720**	**1100**	**0**	**330**	**720**	**1100**
Number of animals/group	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**
Organ weight (g. absolute)								
Kidney	59.17	60.31	62.42	60.90	50.97	52.78	57.24	62.89
Liver	406.47	411.53	444.69	509.90 *	344.04	347.13	402.58	372.87
Thymus	11.12	8.46	15.00	5.15	13.56	12.91	13.80	14.48
Organ weight (adjusted for terminal body weight)								
Terminal bodyweight (kg)	12.0	12.2	11.9	10.7	10.9	11.0	11.2	10.8
Kidney	58.19	58.56	61.69	64.37	51.24	52.81	56.03	63.78**
Liver	398.69	397.66	438.94	537.30 **	345.37	347.27	396.8 *	377.16
Thymus	10.32	7.04	14.41	7.95	13.73	12.93	13.05	15.04
Histopathology incidence								
Liver hepatocyte vacuolation	0	0	0	1	0	0	0	0
Kidney focal nephrosis	0	0	0	0	0	0	0	1
Thymus mild/moderate atrophy	0	0	0	3	0	0	0	0

* Statistically significant difference from controls. $p<0.05$, using Student's t-test or Dunnett's test.

** Statistically significant difference from controls. $p<0.001$, using Student's t-test or Dunnett's test.

Conclusion

The 90-day dog oral NOAEL was 650 mg/kg bw/day. Classification according to Regulation (EC) No 1272/2008 is not required. The endpoint was agreed in the EFSA Conclusion and is still considered valid.

RMS comments and conclusion:

The study was done according to the latest version of OECD 409 (1998) and follows principles of GLP. The observed study deviation from the guideline included no measurements of ornithine decarboxylase (ODC) and no histopathological examination of: bonne marrow, Payers' patches were not specifically mentioned, testes included epididymis. Additionally, to the guideline requirement, pancreas, pituitary gland and prostate gland

were weighted. Despite the small deviations, the study considered to be acceptable for the analysed endpoints. Analysed information relevant for the risk assessment were reported in tabular form.

No death occurred during the study. Clinical signs observed (including vomiting/regurgitation) are not considered treatment related as they were observed also in the control group. One male at 1100 mg/kg bw/day showed signs of idiopathic febrile necrotising arteritis (Beagle Pain Syndrome) form day 64 onwards thus, it received steroid treatment. Findings present only in this mail cannot be directly linked to the treatment with GA4/7.

Body weight gain was slightly decreased compared to the control in both sexes dosed at 1100 mg/kg bw/day and correlates with slightly lower food consumption present in these groups. The effect was not statistically significant.

Table B.6.3.2/02-2 Body weight (kg) and overall weight gain (kg) for male dogs dosed over 13 weeks with GIBBERELLINS GA4/GA7

Week	Males				Females			
	Dose (mg/kg bw/dayay)							
	0	**330**	**720**	**1100**	**0**	**330**	**720**	**1100**
-2	8.4	8.6	8.8	8.6	7.4	7.5	7.5	7.7
-1	8.8	8.9	9.0	8.7	7.7	7.9	7.8	8.1
0	9.0	9.1	9.2	8.8	7.8	8.0	8.1	8.3
1	9.2	9.5	9.5	9.3	8.2	8.4	8.4	8.7
2	9.6	9.9	9.7	9.6	8.5	8.7	8.7	8.8
3	9.8	10.2	10.0	9.6	8.8	8.9	8.9	8.9
4	10.1	10.4	10.1	9.9	8.9	9.1	9.1	9.2
5	10.3	10.7	10.5	10.2	9.3	9.6	9.5	9.5
6	10.4	10.8	10.7	10.2	9.5	9.7	9.5	9.7
7	10.9	11.3	11.0	10.4	9.8	10.0	9.9	10.0
8	11.1	11.4	11.2	10.7	10.0	10.2	10.2	10.1
9	11.4	11.7	11.5	10.9	10.3	10.4	10.4	10.3
10	11.6	12.0	11.7	10.7	10.5	10.4	10.6	10.4
11	11.8	12.2	11.9	10.8	10.6	10.8	10.8	10.5
12	11.8	12.2	11.9	10.7	10.7	11.0	11.1	10.7
13	12.0	12.5	12.1	10.7	11.0	11.1	11.3	10.9
Cumulative gain Day 0 to 90	3.0 (↑33%)	3.4	3.0	1.9 (↑22%)	3.2 (↑41%)	3.2	3.3	2.6 (↑31%)

Small thymus weights were noted in 3/4 ♂ at 1100 mg/kg bw/day which coincided with thymus atrophy found in the same males. Thymus is an organ that is sensitive to the effects of stress (endogenous corticosteroids), general toxicity and aging thus, chemical-induced thymic atrophy from stress-related lymphocyte apoptosis and age-related thymic involution, should be differentiated. Thymus atrophy was found only in males dosed at the highest dose despite low thymus weight was observed also in the low dose group. No dose-response effect was observed. The finding was attributed by the study author to be a secondary effect of stress. According to the » EFSA supporting publication 2015:EN-782« thymus atrophy was frequently associated with significant body weight deficits which is also the case in this study. 1 of the same 3 males having thymus atrophy and receiving steroid treatment showed also adrenal cortical vacuolation and mild adrenal cortical atrophy. The dog was treated with steroidal drugs and consequently findings in this male could not be directly linked to the treatment with GA4

Table B.6.3.2/02-3 Incidence of cortical atrophy at termination

	Males				Females			
Dose level (mg/kg bw/day)	**0**	**330**	**720**	**1100**	**0**	**330**	**720**	**1100**
Number of animals/groups	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**
Histopathology incidence								
Adrenal gland cortical atrophy	0	0	0	2	0	0	0	0

Haematological and urinalyses parameters were considered to be unaffected by the treatment. Clinical chemistry parameters did not show relevant effects which could be attributed to the treatment. The increase in adjusted kidney weight show a dose response both in males and females and when compared to the control at 1100 mg/kg bw/day being: ♂: 11%, ♀: stat. sign. 24%. Increase in absolute and adjusted liver weight in males show a dose response and is statistically significant at the 1100 mg/kg bw/day (25% ↑ absolute liver weight, 35% ↑adjusted liver weight). Histopathological liver and kidney effects were found only in 1 male and 1 female, respectively.

A NOAEL of 700 mg/kg bw/day is proposed based on the reduction in bodyweight gain and food consumption, and effects seen in liver and kidney.

Applicants overall conclusion on short term toxicity studies.

The studies summarised above are considered acceptable for characterising the repeated dose sub-chronic toxicity of gibberellins. Investigations of neurotoxicity, immunotoxicity, genotoxicity (by way of micronuclei formation) and potential hormonal changes were not included in the above studies; all of which are requirements of Regulation (EU) No 283/2013. An acceptable mouse micronucleus test (B.6.4.2/01) is available therefore further genotoxicity testing is not required. In addition, functional behavioural testing was not performed and there were no specific histopathological investigations or *in situ* perfusion, although as there are no structural alerts for neurotoxicity further testing is not proposed. There are no indications of specific immunotoxicity in the standard parameters investigated therefore further testing is not required. Specific investigations of endocrine effects have not been assessed outside of the recommendations of the guideline (OECD 408), however further specific testing is not considered necessary at this time in the absence of any alerts for endocrine disruption. As GA4/7 does not possess immunotoxic effects it therefore meets the criteria for consideration as a low-risk active substance.

RMS comments and conclusion:

The two provided *in vivo* test mouse micronucleus test (B.6.4.2/01, B.6.4.2/02) were found to be as supportive information as the results could not be defined as clearly negative based on the criteria listed in the current version of the guidance OECD (474). Nevertheless, the final conclusion will be reached after the commenting period of the DRAR. The RMS is of the opinion that, overall GA4/7 does not show any specific neurotoxic and/or immunotoxic effects, thus new, additional testing is not considered necessary.

b) <u>Evaluation of additional data for the purpose of renewal of Annex I inclusion</u>

No new 90-day studies were submitted for the purpose of renewal of Annex I inclusion.

B.6.3.3. Other routes

According to Regulation (EU) No 283/2013 the requirement to conduct short-term toxicity studies in other routes is dependent on the likely route of human exposure and the physical-chemical properties of the active

substance. Assessments of repeated-dose toxicity via the inhalation route are not considered necessary as the active substance is not volatile, and acute studies have shown low toxicity via the inhalation route. Assessments of repeated-dose toxicity via the dermal route are not considered necessary based on the low acute dermal toxicity of the substance and the lack of effects seen in oral studies; systemic toxicity following dermal exposure is expected to be lower compared to oral exposure and therefore further testing by the dermal route is not justified.

RMS comments and conclusion:

Agree with the applicant that further testing by other routes is not justified.

B.6.4. GENOTOXICITY

Data on the *in vitro* and *in vivo* genotoxic potential of GA4/7 were submitted during the EU review and are available in the EU DAR. The studies below were considered acceptable in the EFSA conclusion and are considered adequate for supporting renewal of GA4/7; no new genotoxicity studies are submitted. EFSA (2012) concluded that GA4/7 is unlikely to be genotoxic.

B.6.4.1. In vitro studies

B.6.4.1.1. *Bacterial assay for gene mutation (Ames test)*

a) Previous evaluation (2005-2011)

B.6.4.1.1/01

PREVIOUS EVALUATION	This study was evaluated in the DAR and has been considered by EFSA.
Data point addressed:	CA 5.4.1/01 (B.6.4.1.1 DAR)
Author(s) (year):	May K. (1997)
Title:	GA4/7: Bacterial mutation assay
Laboratory report / project number:	96/FNA024/0935
Testing facility:	Huntingdon Life Sciences Ltd., Eye, Suffolk, IP23 7PX, England
Published:	No
Test guideline used:	OECD 471 (1983), EEC Method B.14, EPA FIFRA 125-17, EPA TSCA (798.5265)
Deviations:	A strain to detect cross-linking mutagens was not included, however a clear negative result was obtained in 5 *Salmonella* strains and positive controls confirmed the validity of the assay, therefore the study is considered acceptable
GLP:	Yes
EU Agreed Endpoint:	Not mutagenic

Executive summary

A bacterial reverse mutation assay (Ames test) was conducted with *Salmonella typhimurium* strains TA1535, TA1537, TA1538, TA98 and TA100 and gibberellins GA4/7 (purity 99%; GA4 >90%, batch D105) at concentrations from 5 to 5000 µg/plate in two independent experiments. No increase in revertant colony numbers over control counts were obtained with any of the tester strains in either the presence of absence of metabolic activation (S9 mix). The positive control chemicals induced marked increases in revertant colony numbers with all strains, confirmed sensitivity of the cultures and activity of the S9 mix.

I Materials and Methods

A. Materials:

1.	**Test Material**	Gibberellins GA4/7
	Description	Fine white powder
	Lot/Batch	D105
	Purity	GA4 >90%
	Stability	Assumed to be stable for the duration of the study
2.	**Solvent**	Dimethylsulfoxide (DMSO)
3.	**Controls**	
	Negative	Culture medium
	Solvent/final concentration	DMSO at 0.1 mL/plate
	Positive	N-ethyl-N'-nitro-N-nitrosoguanidine, 9-amidoacridine, 2-nitrofluorene, benzo[a]pyrene and 2-aminoanthracene
4.	**Metabolic activation**	The S9 was prepared in-house from livers of male CD rats treated with a single dose of 500 mg/kg bw AROCLOR 1254 by i.p. injection.
5.	**Bacterial strains**	*Salmonella typhimurium* strains TA1535, TA1537, TA1538, TA98 and TA100

B. Study Design and Methods

1. Preliminary cytotoxicity assay

Six concentrations (5, 15, 50, 150, 500, 1500 and 5000 µg/plate plus the solvent (DMSO) control) of the test substance were assessed for toxicity using the five tester strains, with and without metabolic activation. Negative controls (culture medium only) were also included. The highest concentration was 50 mg/mL in the solvent, providing a final concentration of 5000 µg/plate. The plate incorporation method was used; plates were incubated for 3 days at 37°C. The highest concentration of 5000 µg/plate was selected for the second experiment as no visible thinning of the background lawn was obtained in the first test at this concentration.

2. Mutagenicity assay

The main assay was conducted with *Salmonella typhimurium* strains TA1535, TA1537, TA1538, TA98 and TA100. All strains were checked for the appropriate amino acid requirement, characteristic spontaneous reversion rate and growth inhibition prior to testing. Experiments were conducted, both in the presence and absence of S9 mix prepared from the livers of Aroclor 1254 induced rats. Four concentrations of the test material (50, 150, 500, 1500, 5000 µg/plate) were tested with absolute negative controls, solvent controls and positive controls. All plates were prepared in triplicate, and incubated for 48 hours at 37°C. After incubation, the number of revertant colonies was counted with an automated colony counter. Growth of the background lawn of non-revertant cells on minimal plates was verified.

3. Evaluation criteria

A result was judged positive where a marked increase in the number of revertant colonies compared to the vehicle control was observed.

3.	**Statistics**	Numerical comparison of means only

II Results and Discussion

A.	Preliminary cytotoxicity assay	No toxicity was observed in the preliminary toxicity assay up to and including the maximum concentration tested (5000 µg/plate).
B.	Mutagenicity assay	Sterility check plates and positive controls gave the expected results and the assay was considered to be valid. There were no increases in revertant colony numbers compared to negative controls at any concentration in either experiment, both in the absence and presence of metabolic activation (S9). The test substance was therefore not considered to be mutagenic.

Conclusion

It was concluded that gibberellins GA4/7 is not mutagenic in this bacterial test system, either in the presence or absence of metabolic activation in the strains tested. Classification according to Regulation (EC) No 1272/2008 is not required. The endpoint was agreed in the EFSA Conclusions and is still considered valid.

RMS comments and conclusion:

The study was done according to previous version of the guideline OECD TG 471 (1983) and follows GLP principles. As the older version of the guideline was used, strain having an AT base pair at the primary reversion site which could detect cross-linking agents was not used. Therefore, the study is considered as supportive. The plate incorporation method was used. No cytotoxicity was observed and no material precipitation reported. Results of positive controls chemicals demonstrated the sensitivity of the test. No relevant increase in revertant colony numbers at the tested material doses was observed.

Under the test conditions, GA4/7 is not mutagenic in the tested species. The study is considered as supportive.

B.6.4.1.1/02

PREVIOUS EVALUATION	This study was evaluated in the DAR and has been considered by EFSA.
Data point addressed:	CA 5.4.1/02 (B.6.4.1.1 DAR)
Author(s) (year):	Lawlor T.E. (1988)
Title:	*Salmonella*/mammalian-microsome plate incorporation mutagenicity assay (Ames test) with a confirmatory assay
Laboratory report / project number:	T8201.501014
Testing facility:	Microbiological Associates, Inc., 9900 Blackwell Road, Rockville, MD 20850
Published:	No
Test guideline used:	None, methods based on Ames (1975) and Maron & Ames (1983) and are therefore comparable to OECD 471
Deviations:	The highest concentration is higher than that recommended by OECD 471; however there was no cytotoxicity at this dose and no evidence for mutagenicity. A strain to detect cross-linking mutagens was not included, however a clear negative result was obtained in 5 *Salmonella* strains and positive controls confirmed the validity of

	the assay. The study is considered acceptable
GLP:	Yes
EU Agreed Endpoint:	Not mutagenic

Executive summary

A bacterial reverse mutation assay (Ames test) was conducted with *Salmonella typhimurium* strains TA1535, TA1537, TA1538, TA98 and TA100 and gibberellins GA4/7 (batch 16-213-CD) at concentrations from 667 to 10000 µg/plate. No increase in revertant colony numbers over control counts were obtained with any of the tester strains in either the presence of absence of metabolic activation (S9 mix). Appropriate positive control chemicals induced marked increases in revertant colony numbers with all strains, confirmed sensitivity of the cultures and activity of the S9 mix.

I Materials and Methods

A. Materials:

1. Test Material Gibberellins GA4/7

Description: White powder

Lot/Batch: 16-213-CD

Purity: Not stated

Stability: Assumed to be stable for the duration of the study

2. Solvent Dimethylsulfoxide (DMSO)

3. Controls

Negative: Culture medium

Solvent/final concentration: DMSO at 0.1 mL/plate

Positive: 9-amidoacridine, 2-nitrofluorene, sodium azide and 2-aminoanthracene

4. Metabolic activation The S9 was prepared in-house from livers of male Sprague-Dawley rats treated with a single dose of 500 mg/kg bw AROCLOR 1254 by i.p. injection.

5. Bacterial strains *Salmonella typhimurium* strains TA1535, TA1537, TA1538, TA98 and TA100

B. Study Design and Methods

4. Preliminary cytotoxicity assay

Aliquots of the tester strain and test material or solvent (DMSO) were added to tubes containing molten top agar. For the metabolically activated tests, S9 was also added. 10, 33, 67, 100, 333, 667, 1000, 3333, 6667, 10,000 µg/plate concentrations were tested in presence or absence of S9 with tester strain TA100.

5. Mutagenicity assay

All assays were conducted based on the methods described by Ames *et al* (1975). For the main mutation assay, the tester strains were five histidine auxotrophs of *Salmonella typhimurium*, TA98, TA100, TA1535, TA1537 and TA1538. In general similar procedures were followed for the cytotoxicity and plate incorporation mutation assay. The test substance was tested at 5 dose concentrations (667, 1000, 3333, 6667 and 10000 µg/plate) and appropriate solvent and negative and positive controls were added to the study design. The study was repeated to provide results from two independent tests.

6. Evaluation criteria

Various criteria are presented in the study report to confirm the validity of each stage of the test in terms of tester strain integrity, positive control validity and levels of cytotoxicity. Ames test evaluation is based on two criteria: 1)

does the test material show positive evidence for mutagenicity [do particular tester strains and conditions result in a doubling of the mean number of revertants/plate] and 2) is this response accompanied by a dose-related increase in revertants. Where a dose-response is observed in TA17537 or TA1538 but the increase in mean number of revertants is less than three fold, and then the result must be shown to be reproducible to be taken as a positive response.

3. Statistics Numerical comparison of means only

II Results and Discussion

A. Preliminary cytotoxicity assay — Ten concentrations ranging from 10 to 10000 µg/plate, with or without S9, were evaluated in the standard plate incorporation cytotoxicity assay. No precipitation and no evidence of cytotoxicity were observed up to the limit dose, 10000 µg/plate. The highest dose selected for the mutation assay was therefore the limit dose of 10000 µg/plate and an additional four doses (down to 667 µg/plate) were included to evaluate any dose response.

B. Mutagenicity assay — The number of revertants in the test plates were similar to counts for negative and solvent controls. There were no positive increases in revertant numbers, in either assay, in the presence or absence of S9, up to a dose concentration of 10mg/plate in any of the tester strains. The positive controls responded appropriately with markedly increased revertant values.

Conclusion

It was concluded that gibberellins GA4/7 was not mutagenic in this bacterial test system, either in the presence or absence of metabolic activation in the strains tested. Classification according to Regulation (EC) No 1272/2008 is not required. The endpoint was agreed in the EFSA Conclusions and is still considered valid.

RMS comments and conclusion:

The study was done according to previous version of the guideline OECD TG 471 (1983) and follows GLP principles. As the older version of the guideline was used, strain having an AT base pair at the primary reversion site which could detect cross-linking agents was not used. Therefore, the study is considered as supportive. A plate incorporation method was used. No cytotoxicity was observed and no material precipitation reported. Thus, the choice of the maximum tested dose, 10000 µg/plate, is acceptable. Results of positive controls chemicals demonstrated the sensitivity of the test. No relevant increase in revertant colony numbers at the tested material doses was observed.

Under the test conditions, GA4/7 is not mutagenic in the tested species. The study is considered as supportive.

Table B.6.4.1.1/02-1Summary of results for two independent Ames tests with GA_4A_7 (DAR, 2011)

With or without metabolic activation	**Tester strain**									
	TA98		**TA100**		**TA1535**		**TA1537**		**TA1538**	
	-S9	**+S9**	**-S9**	**+S9**	**-S9**	**+S9**	**-S9**	**+S9**	**-S9**	**+S9**
Dose conc. µg/plate	Mean number of revertants from three replicate plates – Assay 1									
Vehicle/solvent control	18	41	112	122	24	15	9	7	13	27

Gibberellins GA_4A_7										
667	28	45	111	124	23	14	4	8	16	24
1000	29	47	109	108	21	14	10	8	15	24
3333	24	40	98	106	20	12	6	4	16	27
6667	19	36	84	74	15	12	5	7	9	35
10000	15	36	66	36	21	15	2	8	9	22
Positive control										
2 nitrofluorene 3 µg	953	--	--	--	--	--	--	--	1083	--
2-aminoanthracene 2 µg	--	3827	--	4795	--	398	--	700	--	3424
Sodium azide 1 µg	--	--	1114	--	800	--	--	--	--	--
9-aminoacridine 75 µg	--	--	--	--	--	--	1075	--	--	--
With or without metabolic activation	**Tester strain**									
	TA98		**TA100**		**TA1535**		**TA1537**		**TA1538**	
	-S9	**+S9**	**-S9**	**+S9**	**-S9**	**+S9**	**-S9**	**+S9**	**-S9**	**+S9**
Dose conc µg/plate	Mean number of revertants from three replicate plates – Assay 2									
Vehicle/solvent control	20	37	93	122	22	19	6	7	15	33
Gibberellins GA_4A_7										
667	20	39	99	110	21	19	5	8	13	30
1000	22	40	91	122	20	15	6	8	19	23
3333	17	38	95	122	20	14	5	9	14	22
6667	19	32	77	119	20	18	6	9	12	24
10000	14	35	69	115	15	18	4	8	9	22
Positive control										
2 nitrofluorene 3 µg	857	--	--	--	--	--	--	--	1177	--
2-aminoanthracene 2 µg	--	4083	--	4714	--	449	--	643	--	3786
Sodium azide 1 µg	--	--	484	--	308	--	--	--	--	--
9-aminoacridine 75 µg	--	--	--	--	--	--	1142	--	--	--

* significantly different from control, $p<0.05$, by one sided Dunnett's test

** significantly different from control, $p<0.01$, by one sided Dunnett's test

vehicle control

positive control

b) Evaluation of additional data for the purpose of renewal of approval

No additional data was required or submitted for the purpose of renewal of approval of GA4/7.

B.6.4.1.2. *Tests for clastogenicity in mammalian cells*

a) Previous evaluation (2005-2011)

B.6.4.1.2/01 Chromosome aberration test

PREVIOUS EVALUATION	This study was evaluated in the DAR and has been considered by EFSA.
Data point addressed:	CA 5.4.1/03 (B.6.4.1.2 DAR)
Author(s) (year):	Kitching J.D. (1997)
Title:	GA4/7 Metaphase chromosome analysis of human lymphocytes cultured *in vitro*
Laboratory report / project number:	FNA 25/962243
Testing facility:	Huntingdon Life Sciences Ltd., P.O. Box 2, Huntingdon, Cambridgeshire, PE18 6ES, England
Published:	No
Test guideline used:	OECD 473, EEC Method B.10, US EPA Method HG-Chrome in vitro
Deviations:	The study was conducted to an earlier version of the guideline therefore only 100 cells were scored compared to the 300 now recommended. However, two independent experiments were conducted both in the presence and absence of metabolic activation and validity was confirmed using appropriate positive controls therefore the study is considered to be acceptable.
GLP:	Yes
EU Agreed Endpoint:	Not genotoxic

Executive summary

An *in vitro* chromosome aberration test was conducted in human lymphocyte cells with gibberellins GA4/7 (purity 99%; GA4 >90%, batch D105) at concentrations up to 2500 µg/mL without metabolic activation, and up to 4000 µg/mL with metabolic activation. The test substance caused significant increases in the number of chromosome aberrations both in the presence and absence of metabolic activation.

I Materials and Methods

A. Materials:

1. Test Material Gibberellins GA4/7

Description White powder

Lot/Batch D105

Purity GA4 >90%, GA7 and other gibberellins <10%

Stability Assumed to be stable for the duration of the study

2. Solvent Dimethylsulfoxide (DMSO)

3. Controls

Solvent/final concentration	DMSO-62.5 µL/culture
Positive	Ethylmethanesulphonate, cyclophosphamide

4. Metabolic activation Mixed function oxidase systems in the liver of a group of male Sprague-Dawley derived male rats were stimulated by Aroclor 1254, administered as a single intraperitoneal injection in Arachis oil at a dose of 500 mg/kg body weight. On the fifth day after injection, following an overnight fast, the rats were killed and their livers removed aseptically.

5. Test cells Human blood was collected aseptically from healthy male donors, pooled and diluted with RPMI 1640 tissue culture medium.

B. Study Design and Methods

1. Cytogenetic assay

Cultured human lymphocytes, obtained from human blood collected from healthy male donors were stimulated to divide by the addition of phytohaemagglutinin. The cells were exposed to the test substance in the presence and absence of S9 mix derived from the livers of rats induced with Aroclor 1254. The test substance was dissolved in DMSO; the test substance as soluble in DMSO at 400 mg/mL however a precipitate was formed on dosing at 4000 µg/mL (1% v/v in tissue culture medium), therefore this was the highest concentration chosen for testing as it was considered to be the limit of solubility.

In the first assay, after 48 hours culture time, the test substance was added to the lymphocytes give final concentrations of 156.3, 312.5, 625, 1250, 2500, 3000 and 4000 µg/mL. Test substance concentrations, positive controls and solvent controls were cultured in duplicate for each concentration and S9 mix was added to 1 of each duplicate culture. Three hours after dosing the cultures containing the S9 mix were centrifuged and the cell pellets resuspended in fresh medium; the cultures were then incubated for a further 15 hours. The cultures without S9 mix were incubated for 18 hours. The test in the absence of S9 mix was repeated at concentrations of 187.5, 375, 750, 1000, 1500, 2000, 2500, 3000 and 4000 µg/mL. Two hours before the cells were harvested; mitotic activity was arrested by addition of colchicine. After 2 h incubation the cell suspensions were centrifuged, the pellets incubated in hypotonic solution, then centrifuged again and fixed in methanol:glacial acetic acid for 2 hours. Cells were resuspended in fresh fixative and dropped onto slides for Giemsa staining. The proportion of mitotic cells/1000 cells in each culture was recorded; approximately 100 metaphase figures were examined from each culture.

A positive response was observed in the first test, therefore it was deemed not necessary to perform the 42 hour harvest in the second test. Instead cultures were initiated and maintained as for the first test, at the following concentrations: 187.5, 375, 750, 1000, 1500, 2000, 2500, 3000 and 4000 µg/mL without S9; 312.5, 625, 1250, 2500, 3000, 3500 and 4000 µg/mL with S9. Concentrations were tested in duplicate and 100 cells were scored. Positive controls were included to confirm the validity of the test; ethylmethanesulphonate was used in the absence of S9 mix and cyclophosphamide was used in the presence of S9.

2. Evaluation criteria

Mitotic index was determined by counting 1000 cells/culture. From these data, three dose levels were selected for metaphase analysis. 200 cells were scored for structural damage for each treatment.

3. Statistics The number of aberrant metaphase figures in each treatment group, including the positive controls, was compared with the solvent control value using Fisher's test.

II Results and Discussion

A. Metaphase analysis

First test

Toxicity: In the absence of S9, toxicity was evident (reduction in mitotic index to 54% of solvent control) at 3000 µg/mL. However, because of poor morphology of the chromosomes in cultures treated with 1250 µg/mL, the test was repeated and no decrease in mitotic index was seen in the repeat teat at 2500 µg/mL. However, many of the

metaphase figures showed signs of toxicity in the form of overcontracted chromosomes. All higher concentrations together with 2000 and 1500 µg/mL were not analysable due to toxicity. Therefore, concentrations selected for metaphase analysis were 2500, 1000 and 750 µg/mL. In the presence of S9, the toxicity was evident at the maximum achievable concentrate, 4000 µg/mL (reduction in mitotic index to 64% of solvent control). Therefore 4000, 3000 and 1250 µg/mL were selected for metaphase analysis.

Metaphase analysis: The test substance caused a statistically significant increase in the number of metaphase figures with aberrant cells at all concentrations in the absence of S9, and at 4000 µg/mL in the presence of S9. The increase seen at 750 µg/mL the absence of S9 was within the historical control range, however all other increases were outside the historical control range. Both positive control substances demonstrated the expected increases in aberrant cells.

Second test

Toxicity: In the absence of S9, toxicity was evident (reduction in mitotic index to 71% of solvent control) at 3000 µg/mL; however the metaphases were overcontracted and not analysable. At 2500 µg/mL there was no reduction in mitotic index but the cells showed morphological signs of toxicity. Therefore concentrations of 2500, 2000 and 1500 µg/mL were selected for metaphase analysis. In the presence of S9 mix, toxicity was evident (reduction in mitotic index to 70% of solvent control) at 3500 µg/mL; 4000 µg/mL was too toxic for analysis. Therefore concentrations of 3500, 3000 and 2500 µg/mL were selected for metaphase analysis.

Metaphase analysis: The test substance caused a statistically significant increase in the number of metaphase figures with aberrant cells at all concentrations in the presence and absence of S9. All other increases were outside of the range. Both positive control substances demonstrated the expected increases in aberrant cells.

Table B 6.4.1.2/01-1 Summary of toxicity and metaphase analysis data

GA4/7 concentration (µg/mL)	First test			Second test		
	Relative mitotic index (%)	Mean no. aberrant cells (%)		Relative mitotic index (%)	Mean no. aberrant cells (%)	
		-g	+g		-g	+g
Without S9						
0 (DMSO)	100	0.25	0.75	100	0.5	0.75
187.5	111	not analysed		96	not analysed	
375	115	not analysed		102	not analysed	
750	91	3.5*	4.0*	135[a]	not analysed	
1000	102	15.0**	15.0**	135[a]	not analysed	
1500	97[a]	not analysed		179[a]	9.5**	10.5**
2000	115[a]	not analysed		223[a]	8.5**	8.5**
2500	91[a]	46.5**	47.0**	148[a]	14.0**	15.5**
3000	20[a]	not analysed		71[a]	not analysed	
4000	0[a]	not analysed		4[a]	not analysed	
Positive control	-	36.5**	38.0**		9.5**	10.0**
With S9						
0 (DMSO)	100	0.5	0.75	100	1.0	1.25
156.3	89	not analysed		not tested		
312.5	102	not analysed		73	not analysed	
625	102	not analysed		82[b]	not analysed	
1250	80[b]	1.5	2.5	77[b]	not analysed	

2500	71[b]	not analysed		59[b]	5.5**	6.0*
3000	71[b]	0.5	2.0	57[b]	6.0**	6.0*
3500	not tested			70[c]	11.5**	11.5**
4000	64[b]	6.0**	6.0**	30[c]	not analysed	
Positive control	-	25.0**	28.0**		14.0**	14.0**

[a]*: precipitate apparent on dosing, not apparent at end of 18 h treatment period*

[b]*: precipitate apparent on dosing, not apparent at end of 3 h treatment period*

[c]*: precipitate apparent on dosing, still apparent at end of 3 h treatment period*

g: gaps

** p<0.01; ** p<0.001*

Conclusion

It was concluded that the test substance was clastogenic in this test system, both in the presence and absence of metabolic activation. However, cytotoxicity was evident at higher concentrations and precipitates were observed at dosing. It was concluded in the EFSA Conclusion that based on weight of evidence, Gibberellins are unlikely to be genotoxic; the agreed endpoint is still considered valid. Two negative *in vivo* micronucleus tests are available supporting the overall conclusion for a lack of genotoxic potential.

RMS comments and conclusion:

The study was conducted according to OECD 473 (1983) using human lymphocytes and following principles of GLP. A total 200 cells per concentration were scored compared to the 300 recommended in the current OECD 473 (2016) and two out of three experimental conditions were covered (3 hours exposure to GA4/7 with S9 mix and 18 hours exposure to GA4/7 without S9 mix). Two experiments were performed. The laboratory cell cycle time for cultured human lymphocyte was 13 hours, thus the sampling time at 18 hours was adequately chosen. The two deviations mentioned are not seen as real deviations from the current guideline as statistically significant increases in the number of aberrant cells in the absence and presence of S9 mix were in both experiments. All increases, except of that observed at concentration 750 µg/mL (without S9 mix) in the first test lie outside the provided historical control range (0-5.25%), see Table 6.4.1.2/01-2. Historical positive control data were not provided; positive controls produced a statistically significant increase in chromosome aberrations. The concurrent negative controls are considered acceptable for addition to the laboratory historical database, however solvents included in the historical control database are not identified. Cytotoxicity was determined by mitotic index. Material precipitation at the beginning of the test but not at the end, was recorded at different doses. In the study report, there were neither pH measurements provided nor pH changes discussed. According to the criteria of the current OECD 473 the test result is not clearly positive as not all acceptability criteria are fulfilled. However, considering the study involves two assays with the same outcome, we consider the study acceptable.

Table 6.4.1.2/01-2 Summary of Historical control data for human lymphocytes treated with solvent controls from June 1983 until June 1996

		Without gaps				**With gaps**			
	Total no. of cells	**no. of aberrant of cells**	**%mean**	**Range of means (%)**		**no. of aberrant of cells**	**%mean**	**Range of means (%)**	
				min	**max**			**min**	**max**
Without S-9 mix	272501	2848	1.05	0	5.25	3325	1.22	0	6.5
With S-9 mix	292672	3047	1.04	0	5.25	3634	1.24	0	6.25

B.6.4.1.2/02 Chromosome aberration test

PREVIOUS EVALUATION	This study was evaluated in the DAR and has been considered by EFSA.
Data point addressed:	CA 5.4.1/04 (B.6.4.1.2 DAR)
Author(s) (year):	Murli H. (1994)
Title:	Mutagenicity test on gibberellic acid A4A7 measuring chromosomal aberrations in Chinese hamster ovary (CHO) cells with and without metabolic activation with a confirmatory assay with multiple harvests
Laboratory report / project number:	15393-0-437Z
Testing facility:	Hazleton Washington, Inc., 9200 Leesburg Pike, Vienna, Virginia 22182
Published:	No
Test guideline used:	US EPA FIFRA Guideline 152B-17; equivalent to OECD 473
Deviations:	Only 100 cells were scored compared to the 300 now recommended. However, two independent experiments were conducted both in the presence and absence of metabolic activation and validity was confirmed using appropriate positive controls therefore the study is considered to be acceptable.
GLP:	Yes
EU Agreed Endpoint:	Not clastogenic

Executive summary

In an *in vitro* clastogenicity assay, the induction of chromosomal aberrations in Chinese hamster ovary cells, exposed to gibberellins GA4/7 (batch no. 21-018-CD) in the presence and absence of metabolic activation supplied by S9, was investigated. Replicate CHO cell cultures were treated with 262, 655, 1310, 1970 or 2620 µg/mL with or without S9. The non-activated assay was harvested after 24 hours exposure. The activated assays were exposed for 6 hours and harvested 12 or 24 hours later. Chromosomal aberrations were analysed in cultures incubated with the four highest concentrations of the test substance. In two independent assays, gibberellins GA4/7 was considered positive for inducing chromosomal aberrations in Chinese Hamster Ovary cells in the absence of metabolic activation provided by S9, at a concentration causing marked cellular toxicity (2620 µg/mL). The assay in the presence of metabolic activation gave no positive responses. In each case, the sensitivity of the method was adequately demonstrated by the positive control responses.

I Materials and Methods

A. Materials:

1. Test Material Gibberellins GA4/7

Description White powder

Lot/Batch 21-018-CD

Purity Not stated

Stability Assumed to be stable for the duration of the study

2. Solvent Ethanol

3. Controls

Negative	Culture medium
Solvent/final concentration	Ethanol
Positive	Mitomycin C, cyclophosphamide

4. Metabolic activation S9 fraction obtained from liver homogenates from male Sprague-Dawley rat induced with Aroclor 1254 to produce mixed function oxidases.

5. Test cells Replicate cultures of Chinese hamster ovary cells (CHO-WBL) were derived from a permanent cell line and recloned to maintain karyotypic stability. The cell line has an average cycle time of 12 to 14 hours and modal chromosome number of 21. The cells were grown on McCoy's culture medium

B. Study Design and Methods

1. Preliminary cytotoxicity assay

Two preliminary range-finding assays were completed, one with and one without the presence of metabolic activation in the form of S9 to determine the test substance concentrations to be used and the optimal harvest time.

2. Cytogenetic assay

Initial and confirmatory tests were conducted for the main cytogenetic assay. For the non-activated assay, cultures were seeded at 1.2 x 10^6 cells (24 hour assay) or 0.8 x 10^6 cells (48 hour assay). One day after initiation the cells were treated with concentrations of 655, 1310, 1970 or 2620 µg/mL and incubated for 24 or 48 hours at 37°C. For the activated assay, cultures were seeded at 1.5 x 10^6 cells (12 hour assay), 1.2 x 10^6 cells (24 hour assay) or 0.8 x 10^6 cells (48 hour assay). One day after initiation the cells were treated with concentrations of 655, 1310, 1970 or 2620 µg/mL and incubated for 6 hours with S9 at 37°C. 0.1 µg/mL colcemid was administered two and a half hours prior to cell harvest for spindle inhibition. Cells were harvested by mitotic shake-off. 100 cells were examined/replicate culture (200 cells/dose) and were scored for structural and numerical aberrations.

3. Evaluation criteria

100 cells were examined/replicate culture (200 cells/dose) and were scored for structural and numerical aberrations. In the evaluation of chromosomal aberrations the following factors were considered-overall chromosome aberration frequency, percentage of cells with any aberrations, percentage of cells with more than one aberration, evidence for increasing frequency of damage with rising doses (a positive dose relationship).

4. Statistics Data were evaluated for statistical significance at $p<0.01$ using Fischer's Exact Test

II Results and Discussion

A. Preliminary cytotoxicity assay

Toxicity was evident in the culture treated with the highest concentration, 2620 µg/mL, without S9. Floating debris, reduced numbers of visible mitotic cells and an approximate 30% reduction in cell monolayer confluence were apparent. There were no indications of toxicity in other cultures incubated without S9 or in any of the metabolically activated cultures. Cell cycle delay was evident in the non-activated assay at 2620 and to some extent at 873 µg/mL. The mitotic index for cultures treated at 262, 873 or 2620 µg/mL were reduced by 17, 55 or 68% respectively in comparison with controls and the respective cell counts were also reduced; 50%, 73% and 61%. In activated cultures the delay in cell cycle was slight and did not merit a change in harvest time. There were no reductions in mitotic index or cell counts.

B. Cytogenetic assay

The concentrations tested were 262, 655, 1310, 1970 and 2620 µg/mL; harvest times were 24.25 and 48 hours. Examination of slides indicated the 1970 and 2620 µg/mL cultures had many metaphases of poor morphology, indicative of toxicity. Significant, concentration-related, increases in the number of cells with chromosomal aberrations were evident at 1970 and 2620 µg/mL. At the 48 hour harvest, chromosomal aberrations were analysed for cultures treated at 262, 655 or 1310 µg/mL. At 1310 µg/mL there was a significant increase in the number of

cells which exhibited chromosomal aberrations. In the initial 12 and 24 hour assay with S9, there were no effect on the incidence of cells with chromosomal aberration or polyploidy at any of the dose concentrations analysed. In the 24.25 hour confirmatory assay with S9, chromosomal aberrations were analysed for four concentrations, 655, 1310, 1970 and 2620 µg/mL and there were no significant increases in cells with aberrations or polyploidy in any of the cultures except the highest concentration. A weakly significant effect was apparent in the 2620 µg/mL cultures. However, since this was not seen in the initial assay or at 48 hours in the confirmatory assay, it was not considered a reproducible result. In the 48 hour assay chromosomal aberrations were analysed for four concentrations, 655, 1310, 1970 and 2620 µg/mL and there were no significant increases in cells with aberrations or polyploidy in any of the cultures.

Table B 6.4.1.2/02-1 Summary of findings in Trials 1 (24 h) and 2 (24.5 h) without metabolic activation (continuous treatment)

Treat-ment	µg/ mL	Tri-al	Cells scor e-d	Cells showing structural chromosomal aberrations from replicate plate counts (%)										
				Endop -loid	Polyp-loid	Judge-ment	Gap	Chromatid type		Chromoso-me type		Other	Total		Judge-ment
							g	ctb	cte	csb	cse	GT	-g	+g	
Medium	-	1	200	0.0	3..0	-	--	--	--	0.5	--	--	0.5	0.5	-
Solvent	10	1	200	0.0	4.0		--	--	--	--	--	--	0.0	0.0	
MMC	0.08	1	25	0.0	2.0	-	4.0	8.0	48.0	4.0	4.0	--	52.0	52.0	+
Test material	655	1	200	0.0	2.5	-	--	--	--	--	--	--	0.0	0.0	-
	1310	1	200	0.5	3.5	-	1.5	2.0	--	--	--	0.5	2.5	4.0	-
	1970	1	200	0.5	4.0	-	3.5	5.0	0.5	--	--	--	5.5	8.5	+
	2620	1	200	0.5	4.0	-	12.0	18.0	--	0.5	--	--	18.5	27.0	+
Medium	-	2	200	0.0	1.0	-	--	0.5	--	0.5	--	--	1.0	1.0	-
Solvent	10	2	200	0.0	3.0		--	--	--	0.5	0.5	--	1.0	1.0	
MMC	0.08	2	25	0.0	1.0	-	12.0	--	16.0	16.0	--	--	28.0	36.0	+
Test material	655	2	200	0.0	1.0	-	0.5	--	--	0.5	--	--	0.5	1.0	-
	1310	2	200	1.0	2.5	-	2.0	2.0	0.5	1.0	--	--	3.5	5.0	-
	1970	2	200	0.0	1.0	-	1.5	4.0	-	1.5	1.0	--	6.5	7.0	+
	2620	2	175	0.5	1.0	-	8.0	26.9	0.6	4.0	--	--	28.0	30.3	+

g gap; ctb chromatid break; cte chromatid exchange; csb chromosome break; cse chromosome exchange; GT fragment, + p<0.1

Conclusion

It was concluded that the test substance was clastogenic in this test system, both in the presence and absence of metabolic activation. However effects were seen at cytotoxic concentrations and it was concluded in the EFSA Conclusions that based on weight of evidence, Gibberellins are unlikely to be genotoxic; the agreed endpoint is

still considered valid. Two negative *in vivo* micronucleus tests are available supporting the overall conclusion for a lack of genotoxic potential.

RMS comments and conclusion:

The study was conducted according to OECD 473 (1983) using Chinese hamster ovary (CHO) cells and following principles of GLP. A total 200 cells/dose were scored compared to the 300 recommended in the current OECD 473 (2016). The required three experimental conditions, with short incubation time in presence and absence of S9 mix and continuous 1.5 cycle length exposure (with additional 12h (with S9 mix) and 48 h (with and without S9 mix) sampling) were tested. The study authors noted a delay in cell cycle time of treated cells durin the range-finding study. According to this finding, a sampling time of 24 hours was decided. Historical positive and negative control data were not reported. The concurrent positive historical produced a statistically significant increase in chromosome aberrations. Cytotoxicity was determined by mitotic index in the range finding experiment, but not in the main experiments. In the study report, there were neither pH measurements provided nor pH changes discussed. In the first Trial (Table B 6.4.1.2/02-1) toxicity was defined as "presence of many metaphases of poor morphology" at 2620 µg/mL. Precipitates were observed for 3.5 hours after dosing at 1970 and 2620 µg/mL. Significant dose related increase in the number of cells with CA were observed at 1970 and 2620 µg/mL. Due to the study result, a confirmatory Trial 2 was performed (see Table B 6.4.1.2/02-1 and Table B 6.4.1.2/02-2). In the second trial at 24.25 h sampling (Table B 6.4.1.2/02-1), toxicity as "presence of many metaphases of poor morphology »was defined at the two highest doses tested, 1970 at 2620 µg/mL. At 48 h sampling (Table B 6.4.1.2/02-2), toxicity was observed at doses, 1310, 1970 and 2620 µg/mL. At 1310 µg/mL the toxicity was defined by presence of sparse numbers of metaphases and many metaphases of poor morphology. Significant increase in the number of cells with CA were observed at 1970 and 2620 µg/mL. In the further test with S9 mix precipitates were observed after dosing at 1970 and/or 2620 µg/mL but not observed at sampling time except at 2620 µg/mL (sampling at 24.25h). There was an increase in the number of cells with CA at the highest dose (se Tables 6.4.1.2/02-5) which was not observed in the first trial, thus not being reproducible.

According to the criteria of the current OECD 473 the test result is not clearly positive as not all acceptability criteria are fulfilled. Considering the study covers two trials with the same outcome, we found the study acceptable. The increases in number of cells with CA in trials without metabolic activation were observed at doses showing considerable cytotoxicity, thus the response of GA4/7 in the tested condition is considered to give equivocal results.

Table 6.4.1.2/02-2 Summary of findings in Trial 2 (48 h) without metabolic activation (continuous treatment)

Treat-ment	µg/ mL	Tri-al	Cells scor e-d	Cells showing structural chromosomal aberrations from replicate plate counts (%)										
				Endop -loid	Polyp-loid	Judge-ment	Gap	Chromatid type		Chromoso-me type		Other	Total		Judge -ment
							g	ctb	cte	csb	cse	GT	-g	+g	
Medium	--	2	200	0.0	1.0	-	--	--	--	0.5	--	0.5	1.0	1.0	-
Solvent	10	2	200	0.0	1.5		--	0.5	--	--	--	--	0.5	0.5	
MMC	Not tested														
Test material	262	2	200	0.0	1.0	-	--	0.5	--	0.5	--	--	1.0	1.0	-
	655	2	200	0.0	2.5	-	--	0.5	--	--	--	--	0.5	0.5	-
	1310	2	200	0.0	3.0	-	4.0	4.5	0.5	0.5	1.0	--	6.5	9.5	+
	1970	2	200	0.0	toxic	-	toxic	toxic	toxic	toxic	toxic	toxic	toxic	toxic	

g gap; ctb chromatid break; cte chromatid exchange; csb chromosome break; cse chromosome exchange; GT fragment, + p<0.1

Table 6.4.1.2/02-3 Summary of findings in Trial 3 (24.25 h) without metabolic activation (6-hour treatment)

Treat-ment	µg/ mL	Tri-al	Cells score -d	Cells showing structural chromosomal aberrations from replicate plate counts (%)										
				Endop-loid	Poly p-loid	Judge-ment	Gap	Chromatid type		Chromoso-me type		Other	Total		Judge-ment
							g	ctb	cte	csb	cse	GT	-g	+g	
Medium	--	2	200	1.5	3.5	-	--	--	--	--	--	--	0.0	0.0	-
Solvent	10	2	200	4.5	6.5		--	--	--	0.5	--	--	0.5	0.5	
MMC	Not tested														
Test material	655	2	200	1.5	1.0	-	--	--	--	--	0.5	--	0.5	0.5	-
	1310	2	200	0.0	1.0	-	--	0.5	--	--	0.5	--	1.0	1.0	-
	1970	2	200	3.0	0.5	-	--	--	--	0.5	--	--	0.5	0.5	-
	2620	2	200	0.0	1.5	-	--	--	0.5	--	0.5	--	toxic	toxic	-

g gap; ctb chromatid break; cte chromatid exchange; csb chromosome break; cse chromosome exchange; GT fragment, + p<0.1

Table 6.4.1.2/02-4 Summary of findings in Trial 1 (12 h) with metabolic activation (6-hour treatment)

Treat-ment	µg/ mL	Tri-al	Cells scor e-d	Cells showing structural chromosomal aberrations from replicate plate counts (%)										
				Endop -loid	Polyp-loid	Judge-ment	Gap	Chromatid type		Chromoso-me type		Other	Total		Judge -ment
							g	ctb	cte	csb	cse	GT	-g	+g	
Medium	-	1	200	0.0	0.5	-	1	--	--	0.5	--	--	0.5	1.5	-
Solvent	10	1	200	0.0	3.0		--	0.5	1	--	0.5	--	2.0	2.0	
CP	0.08	1	25	2.0	3.0	-	16.0	--	12.0	--	8.0	--	20.0	24.0	+
Test material	655	1	200	0.0	1.0	-	--	1.0	1.0	0.5	0.5	--	2.5	2.5	-
	1310	1	200	0.0	1.0	-	0.5	2.0	1.0	--	0.5	--	3.5	4.0	-
	1970	1	200	0.5	1.0	-	0.5	1.0	1.0	--	--	--	2.5	3.0	-
	2620	1	200	1.5	5.0	-	2.0	1.5	2.5	1.0	0.5	--	5.0	6.0	-

g gap; ctb chromatid break; cte chromatid exchange; csb chromosome break; cse chromosome exchange; GT fragment, + p<0.1

Table 6.4.1.2/02-5 Summary of findings in Trial 2 (24 h) and Trial 3 (24.25 h) with metabolic activation (6-hour treatment)

Treat-ment	µg/ mL	Tri-al	Cells scor e-d	Cells showing structural chromosomal aberrations from replicate plate counts (%)										
				Endop -loid	Polyp-loid	Judge-ment	Gap	Chromatid type		Chromoso-me type		Other	Total		Judge -ment
							g	ctb	cte	csb	cse	GT	-g	+g	
Medium	-	1	200	0.0	3.0	-	--	--	0.5	--	--	--	0.5	0.5	-
Solvent	10	1	200	0.5	0.0		--	--	--	0.5	0.5	--	1.0	1.0	
CP	0.08	1	25	0.0	0.0	-	--	28	44.0	44.0	--	36	96.0	96.0	+
Test material	655	1	200	0.0	2.0	-	--	1.0	2.0	0.5	0.5	--	2.5	2.5	-
	1310	1	200	0.0	1.0	-	1.5	2.0	2.0	1.0	1.0	--	5.5	7.0	-
	1970	1	200	0.5	1.5	-	--	1.0	1.0	1.5	--	--	3.0	3.0	-
	2620	1	200	0.0	0.5	-	1.5	1.5	2.0	0.5	--	--	2.5	3.5	-
Medium	-	1	200	1.0	2.0	-	--	--	0.5	0.5	1.5	--	1.5	1.5	-
Solvent	10	1	200	3.0	1.0		--	--	--	0.5	0. 5	--	1.0	1.0	
CP	0.08	1	25	0.0	--	-	--	36.0	76.0	44.0	--	16	96.0	96.0	+
Test material	655	1	200	2.5	0.5	-	1.5	--	--	0.5	--	--	0.5	1.0	-
	1310	1	200	0.0	1.5	-	--	--	0.5	2.0	0.5	--	3.0	3.0	-
	1970	1	200	0.0	2.5	-	1.5	0.5	2.5	2.5	0.5	--	5.5	6.0	-
	2620	1	200	0.5	3.5	-	--	2.5	3.5	3.0	0.5	--	8.0	8.0	+

g gap; ctb chromatid break; cte chromatid exchange; csb chromosome break; cse chromosome exchange; GT fragment, + p<0.1

Table 6.4.1.2/02-6 Summary of findings in Trial 3 (48 h) with metabolic activation (6-hour treatment)

Treat-ment	µg/ mL	Tri-al	Cells scor e-d	Cells showing structural chromosomal aberrations from replicate plate counts (%)											
				Endop -loid	Polyp-loid	Judge-ment	Gap	Chromatid type		Chromoso-me type		Other	Total		Judge -ment
							g	ctb	cte	csb	cse	GT	-g	+g	
Medium	-	1	200	0.0	1.0	-	0.5	--	--	--	--	--	0.0	0.5	-
Solvent	10	1	200	0.0	0.0		1.5	--	0.5	0.5	0.5	--	1.0	2.5	
CP	Not tested														
Test material	655	1	200	0.0	1.0	-	0.5	--	--	0.5	0.5	--	1.0	1.5	-
	1310	1	200	0.0	0.0	-	0.5	--	0.5	--	--	--	0.5	1.0	-
	1970	1	200	0.0	0.5	-	--	--	--	0.5	1.5	--	2.0	2.0	-
	2620	1	200	0.0	0.0	-	--	--	2.0	0.5	--	--	0.5	0.5	-

g gap; ctb chromatid break; cte chromatid exchange; csb chromosome break; cse chromosome exchange; GT fragment, + p<0.1

b) Evaluation of additional data for the purpose of renewal of approval

No additional data was required or submitted for the purpose of renewal of approval of GA4/7.

B.6.4.1.3. *Tests for gene mutations in mammalian cells*

a) Previous evaluation (2005-2011)

B.6.4.1.3/01 The mouse lymphoma assay (MLA)

PREVIOUS EVALUATION	This study was evaluated in the DAR and has been considered by EFSA.
Data point addressed:	CA 5.4.1/05 (B.6.4.1.3 DAR)
Author(s) (year):	Lloyd J.M. (1997)
Title:	GA4/7: Mammalian cell mutation assay
Laboratory report / project number:	96/FNA026/0944
Testing facility:	Huntingdon Life Sciences Ltd., Eye, Suffolk, IP23 7PX, England
Published:	No
Test guideline used:	OECD 476, EEC Directive 88/302 Part B, US EPA (Method HG-Gene Muta-Somatic Cells)
Deviations:	The study was conducted to an earlier version of the guideline, however there are no major deviations and the study is considered to be acceptable
GLP:	Yes

EU Agreed Endpoint:	Not mutagenic

Executive summary

An *in vitro* mammalian cell gene mutation test was conducted in L5178Y mouse lymphoma cells with gibberellins GA4/7 (purity 99%; GA4 >90%, batch D105) at concentrations from 500 to 3000 µg/mL, both with and without metabolic activation. Concentrations of the test substance for the main assay were selected based on the results of a preliminary assay. In the main assay, cell cultures were exposed to five concentrations of the test material in the presence and absence of S9 mix for 3 hours, followed by a 48 hour expression period. Cultures exposed to gibberellins GA4/7 showed no increases in TK-/- mutant colony numbers and there were no significantly increased mutant frequencies compared to solvent controls.

I Materials and Methods

A. Materials:

1.	**Test Material**	Gibberellins GA4/7
	Description	Fine white powder
	Lot/Batch	D105
	Purity	GA4 >90%, GA7 and other gibberellins <10%
	Stability	Assumed to be stable for the duration of the study
2.	**Solvent**	DMSO
	Solvent/final concentration	DMSO
	Positive	Methylmethanesulphonate, 20-methylcholanthrene
4.	**Metabolic activation**	S9 fraction obtained from liver homogenates from male CD rats induced with Aroclor 1254 to produce mixed function oxidases.
5.	**Test cells**	The L5178Y TK+/- mouse lymphoma cells used were periodically cleansed with a thymidine, hypoxanthine, methotrexate and glycine solution in order to kill spontaneous TK-deficient revertants. Cells were also regularly screened for mycoplasma infection.

B. Study Design and Methods

1. Preliminary cytotoxicity assay

The test substance was soluble in DMSO up to a maximum concentration of approximately 433 mg/mL. A preliminary range finding test was conducted to determine appropriate concentrations for use in the main test. The highest concentration chosen for the preliminary test was based on the maximum solubility. Test concentrations were 0 (solvent control), 7.81, 15.63, 31.25, 62.5, 125, 250, 500, 1000, 2000 and 4300 µg/mL. L5178Y cells were added to the assay mixture as a suspension of 3 mL containing 2 x 10^6 cells/mL (totalling 6 x 10^6 cells) in R10P medium. Cells were cultured in the absence and presence of S9 mix obtained from the livers of Aroclor 1254 induced rats. Cultures were incubated in a shaking water bath at 37°C for 3 hours. After treatment, the cells were centrifuged, washed and resuspended in fresh medium then incubated in 5% CO_2 in air at 37°C. After 24 and 48 hours, the cell concentrations of each culture were measured using a coulter counter; cell concentrations were adjusted after culture for 24 hours to approximately 2 x 10^5 cells/mL.

2. Cytogenetic assay

For the main assay, exponentially growing L5178Y cells were adjusted to a concentration of 2 x 10^6 cells/mL in R10P medium. The assay mixtures each contained 6 mL (1.2 x 10^7 cells). Test concentrations in the first assay were 0 (solvent control), 500, 1000, 1500, 2000 and 3000 µg/mL and in the second assay were 0 (solvent control), 1000,

1500, 2000, 2500 and 3000 µg/mL. Both assays were conducted in the presence and absence of metabolic activation (S9). The positive control in the absence of S9 mix was methylmethanesulphonate, and in the presence of S9 was 20-methylcholanthrene. All treatments were cultured in duplicate. Cultures were incubated at 37°C for 3 hours in a shaking water bath. After treatment, the cells were centrifuged, washed and resuspended in fresh medium then incubated in 5% CO_2 in air at 37°C. After 24 and 48 hours, the cell concentrations of each culture were measured using a coulter counter; cell concentrations were adjusted after 24 hours to 2 x 10^5 cells/mL. At the end of the 48 hour expression time the cells were added to cloning medium containing the selective agent TFT. Cell viability was estimated by adding the cell suspension to cloning medium without TFT. The cultures were plated and incubated at 37°C for 10-12 days, after which individual cells were counted.

3. Evaluation criteria

The criteria for a positive response were: an increase in mutant frequency in treated cultures of at least 100 relative to the concurrent control; the demonstration of a statistically significant increase in mutant frequency following treatment with the test substance; evidence of a dose relationship over at least two dose levels, in any increase in mutant frequency; demonstration of reproducibility in any increase in mutant frequency; increases in absolute mutant colony numbers in treated cultures; cultures showing an increase in mutant frequency should have total growth values of not less than 10%.

4. Statistics Weighted analysis of variance

II Results and Discussion

A. Preliminary cytotoxicity assay

Colour changes were observed in the media of cultures treated at concentrations of 1000 µg/mL and above, but these were not related to concurrent changes in pH. Slight precipitation of the test substance was observed at 2000 µg/mL, with definite precipitation at 4300 µg/mL. There was no evidence of toxicity to cultures in the absence of S9 up to and including 1000 µg/mL, but survival was reduced to 36.8% and 5.3% of the solvent control value at 2000 and 4300 µg/mL, respectively. Similarly, there was no evidence of toxicity to cultures in the presence of S9 up to and including 1000 µg/mL, but survival was reduced to 41.1% and 12.3% of the solvent control value at 2000 and 4300 µg/mL, respectively.

B. Mutation assay

In both experiments, colour changes (as seen in the preliminary test) in treated cultures were observed at 1000 µg/mL and above. There was slight precipitation of the test substance at concentrations of 1500 and 2000 µg/mL, with definite precipitation observed at 2500 and 3000 µg/mL.

Without S9: In the first assay without S9, total growth was reduced to 16.9% of the solvent control at 3000 µg/mL. In the second assay without S9 there was evidence of a dose-related response, but total growth was reduced to 42.8% of the solvent control at 3000 µg/mL. In both the first and second assay there was no significant increase in mutation frequency (per 10^6 survivors) or in mutant colony numbers at any concentration.

With S9: In both assays no decreases in total growth were observed between 500 and 2000 µg/mL in the first assay, and between 1000 and 2500 µg/mL in the second assay. At 3000 µg/mL, total growth values were reduced to 19.0 and 49.6% in the first and second assay, respectively. It was noted that the solvent control suspension growth values, particularly in the second assay, were uncharacteristically low compared with the treated cultures. In both the first and second assay there was no significant increase in mutation frequency (per 10^6 survivors) or in mutant colony numbers at any concentration.

Conclusion

It was concluded that the test substance was not mutagenic in the mouse lymphoma forward mutation assay, either in the presence or absence of metabolic activation. The sensitivity of the method was adequately demonstrated by the positive control responses. The endpoint was agreed in the EFSA Conclusions and is still considered valid.

RMS comments and conclusion:

The study was conducted according to OECD 476 (1984) using mouse lymphoma L5178Y cells and following principles of GLP. The mouse lymphoma assay (MLA) and TK6 test using the thymidine kinase (TK) locus were originally contained in Test Guideline 476 adopted in 1984 and revised in 1997. New internationally harmonized recommendations for assay acceptance criteria and data interpretation for the MLA were developed and from July 2015 incorporated into the new Test Guideline (TG 490). The current version is from 2016. The test was conducted with/without /-S9 mix in two experiments with 5 concentrations in duplicate (soft agar method). The highest tested concentration was selected considering the cytotoxicity, falling in the criteria between 20 and 10% RTG. Slight precipitate was seen at 1500 and 2000 µg/mL with define precipitation at 2500 and 3000 µg/mL and consequently leaving only two concentrations (500 and 1000 µg/mL) where no precipitate was observed. No distinction between small and large colonies was made. Adequate number of cells was analysed. Historical control data for negative and positive controls were not reported, and not considered necessary due to the evaluation and interpretation criteria for MLA according to OECD 490 (2016). The MF of the concurrent solvent control in the first experiment was 94.8-119.5 x 10^{-6}, CE was 81-92.5 %, SG was 9.8 fold without S9 mix and **7.01 fold with S-9 mix** after 3 hours treatment. The MF of the concurrent solvent control in the second experiment was 84.5 x 10^{-6} without S9 mix and **181 x 10^{-6} with S-9 mix**, CE was 68-76.9 %, SG was 20.93 fold without S-9 mix and **5.93 fold with S-9 mix** after 3 hours treatment. The fulfilment of acceptance criteria of the positive control could not be confirmed, as in the experiments the size of colonies were not reported. The IMF for the positive control **without S-9 mix** was **below 300 x 10^{-6}** in both experiments, and with S-9 mix above 300 x 10^{-6}. Values in bold do not fulfil the acceptability criteria. The IMF of the treated cultures were compared to Global Evaluation Factor (GEF) from the OECD 490 (2016), 90x10^{-6} which was not exceeded for the treated cultures in all experiments. The mutant frequency of the solvent control in the second experiment was extremely high compared to the values of the treated cultures. In all the experimental conditions examined, there was no concentration related response.

GA4/7 is not considered clearly negative as not all acceptability criteria were fulfilled. Additionally, only two concentrations were without precipitate thus, the study is considered as supportive.

Table 6.4.1.3/01-1 Summary of results without S-9 mix

Table 1: Summary of results without S-9 mix

Expression time	Test 1			Test 2		
	Dose µg/ml	Mean % RTG	Mutant frequency per 10^6 survivors	Dose µg/ml	Mean % RTG	Mutant frequency per 10^6 survivors
48 hours	0	100	95	0	100	85
	500	93	73	1000	85	77
	1000	87	90	1500	74	84
	1500	68	107	2000	67	99
	2000	76	78	2500	56	101
	3000	17	79	3000	43	92
	MMS	50	***326	MMS	59	***331

*** $p<0.001$

Table 6.4.1.3/01-1 Summary of results with S-9 mix

Table 2: Summary of results with S-9 mix

Expression time	Test 1			Test 2		
	Dose µg/ml	Mean % RTG	Mutant frequency per 10^6 survivors	Dose µg/ml	Mean % RTG	Mutant frequency per 10^6 survivors
48 hours	0	100	120	0	100	181
	500	146	116	1000	227	104
	1000	137	107	1500	202	115
	1500	137	113	2000	227	99
	2000	123	86	2500	135	116
	3000	19	100	3000	50	142
	20-MC	50	***580	20-MC	47	***504

*** $p<0.001$

B.6.4.1.3/02 The mouse lymphoma assay (MLA)

PREVIOUS EVALUATION	This study was evaluated in the DAR and has been considered by EFSA.
Data point addressed:	CA 5.4.1/06 (B.6.4.1.3 DAR)
Author(s) (year):	Cifone M.A. (1994)
Title:	Mutagenicity Test on Gibberellic Acid A_4A_7 in the L5178Y TK+/- Mouse Lymphoma Forward Mutation Assay with an Independent Repeat
Laboratory report / project number:	HWA 15693-0-431
Testing facility:	Hazleton Washington, Inc., 9200 Leesburg Pike, Vienna, Virginia 22182
Published:	No
Test guideline used:	US EPA FIFRA Guideline 152B-17
Deviations:	The study is broadly comparable to the OECD guideline; appropriate controls confirmed the validity of the assay and the study is considered to be acceptable
GLP:	Yes
EU Agreed Endpoint:	Not mutagenic

Executive summary

In a mammalian cell cytogenetics assay the ability of gibberellins GA4/7 (purity 87.3%, batch 21-018-CD) to induce forward mutations at the thymidine kinase (TK) locus in the mouse lymphoma cell line L5178Y was

evaluated. In the assay without S9, concentrations of 218-1570 µg/mL were tested without observing any concentration-related increase in mutant frequency. In the assay with S9, doses from 218 to 1530 µg/mL were tested, also without observing any dose-related increase in mutant frequency.

I Materials and Methods

A. Materials:

1.	**Test Material**	Gibberellins GA4/7
	Description	White powder
	Lot/Batch	21-018-CD
	Purity	87.3%
	Stability	Assumed to be stable for the duration of the study
2.	**Solvent**	1% ethanol
3.	**Controls**	
	Negative	Culture medium
	Solvent/final concentration	DMSO
	Positive	Methylmethanesulphonate, 20-methylcholanthrene
4.	**Metabolic activation**	S9 fraction obtained from liver homogenates from male Sprague-Dawley rats induced with 500 mg/kg bw Aroclor 1254.
5.	**Test cells**	L5178Y mouse lymphoma cells, heterozygous at the TK locus, designated clone 3.7.2c, obtained as stock cultures from Burroughs Wellcome Company

B. Study Design and Methods

1. Preliminary cytotoxicity assay

Gibberellins GA4/7 was soluble in culture medium up to 1200 µg/mL. The test substance was dissolved in ethanol up to 262 mg/mL and preliminary cytotoxicity investigations completed to determine toxicity to cells in presence or absence of metabolic activation. No or only weak toxic effects were apparent up to 873 µg/mL so the mutation assay was initiated with doses up to 2620 µg/mL to elicit a broad range of toxic effects.

2. Mutation assay

Mutagenicity testing involved two independent assays, one involving metabolic activation and the second without activation. Cells from laboratory stock cultures were seeded at 6 x 10^6/tube in culture medium, centrifuged, re-suspended in treatment medium, incubated for 4 hours and then, after washing, were re-suspended in growth medium. The non-activation assay involved three vehicle controls, two positive controls and up to nine different test concentrations (one culture/dose level), with several treated cultures eliminated during the expression phase. A 2-day expression phase allowed recovery, growth and expression of the $TK^{-/-}$ phenotype. Cell densities were counted 24 hours after treatment and adjusted to 3 x 10^5 cells/mL. On day 2, cell counts were determined again and cultures selected for cloning and mutant selection. Where possible, at least five doses were selected to include a range of non-toxic to highly toxic (10-20% relative growth) treatments. Three plates were prepared at each dose-1 x 10^6 cells suspended in selection media/dish. Cloning efficiency was determined by serially diluting the cells and seeding three dishes with 200 cells in cloning medium and incubating for 10 to 14 days in a 5% CO_2:95% air atmosphere. An automatic colony counter was used to count the colonies and mutant frequency was calculated as the ratio of total number of mutant colonies to total number of seeded cells. Treatment related toxicity was determined by the relative suspension growth over the expression period multiplied by the relative cloning efficiency. The activated assay was conducted as described for the non-activated assay except different positive controls were used and S9 and the necessary co-factors were include in the medium during the 4-hour treatment phase.

3. Evaluation criteria

Cell cultures are exposed to a range of test concentration intended to span responses from non- to highly toxic. An increase in mutation frequency and a series of defined assay evaluation criteria take into account variable mutation frequencies at highly toxic doses. The minimum test criterion used to determine if a given treatment is mutagenic is a mutant frequency that is ≥2-fold the concurrent background frequency (defined as the average mutant frequency of vehicle control cultures). Since the observation of a mutant frequency that meets the minimum criterion in a single treatment within a range of test concentration is not deemed sufficient evidence to define the test material as a mutagen, the Study Director has a range of other criteria to be applied on a case-by-case basis to each test scenario, some of which are outlined in the study report but are not presented in this summary because they do not apply.

4. Statistics Not required beyond numerical comparison

II Results and Discussion

A. Preliminary cytotoxicity assay

Ten doses of gibberellins GA4/7 ranging from 1.70 to 873 µg/mL, with or without S9, were evaluated in mouse lymphoma cells and indicated that the test material was only weakly toxic. Cytotoxicity was evident in the absence of S9 at the high dose (873 µg/mL) only. In the presence of S9, doses of 873 and 437 µg/mL showed evidence of cytotoxicity but lower doses were non-toxic. Since the highest dose used in the preliminary range-finder was only weakly toxic the dose concentration was increased for the main assays and gibberellins GA4/7 was administered at up to 2620 µg/mL.

B. Mutation assay

Under both non-activated and activated conditions three mutation assays were trialed. In both cases the first trial was unacceptable due to highly variable background mutation frequency. For the non-activated assay, eight doses, ranging from 218 to 2620 µg/mL, were tested in the second trial. The three highest doses were cytotoxic. The lower five doses induced relative growths of 105.9 to 43.3% and were analysed for induction of mutations. In the third trial, nine doses (437 to 2270 µg/mL) were selected. The four highest doses caused excessive toxicity and the lower five doses resulted in relative growth of 81.3 to 16.4% and were analysed for induction of mutations. In both trials the mutation frequencies for analysed test concentrations were similar to the vehicle controls. None of the treatments resulted in a mutant frequency that exceeded the minimum criterion to be considered a positive response. For the activated assay, six doses, ranging from 218 to 1750 µg/mL, were tested in the second trial. The highest dose was cytotoxic. The lower five doses induced relative growths of 72.4 to 11.6% and were analysed for induction of mutations. In the third trial, nine doses (218 to 1750 µg/mL) were selected. The four highest doses caused excessive toxicity or reduced percent relative growth to <10% and were considered unsuitable for analysis. The lower five doses resulted in relative growth of 45.4 to 16.1% and were analysed for induction of mutations. None of the treatments resulted in a mutant frequency that exceeded the minimum criterion to be considered a positive response. In all cases the positive controls resulted in mutant frequencies that greatly exceeded the minimum criterion.

Conclusion

It was concluded that the test substance was not mutagenic in the mouse lymphoma forward mutation assay, either in the presence or absence of metabolic activation. The sensitivity of the method was adequately demonstrated by the positive control responses. The endpoint was agreed in the EFSA Conclusions and is still considered valid.

RMS comments and conclusion:

The study was conducted according to OECD 476 (1984) using mouse lymphoma L5178Y cells and following principles of GLP. The mouse lymphoma assay (MLA) and TK6 test using the thymidine kinase (TK) locus were originally contained in Test Guideline 476 adopted in 1984 and revised in 1997. New internationally harmonized recommendations for assay acceptance criteria and data interpretation for the MLA were developed and from July 2015 incorporated into the new Test Guideline (TG 490). The current version is from 2016. The

test was conducted with/without /-S9 mix in three experiments (2 analysable) with 5 concentrations in triplicates (soft agar method). The top concentration was adequately selected (except in experiment 2 without S-9 mix), fulfilling the cytotoxicity criteria, between 20 and 10% RTG. No precipitate was observed during the experiment, test on solubility was done before the start of the experiment. No distinction between small and large colonies was made. Adequate number of cells was analysed. Historical control data for negative and positive controls were not reported, and not considered necessary due to the evaluation and interpretation criteria for MLA according to OECD 490 (2016). The MF of the concurrent vehicle control in the second experiment was **28 x 10^{-6} without** S9 mix and 36 x 10^{-6} with S9 mix, CE was 89-93 %, SG was **34 fold without S9** mix and 26 fold with S-9 mix after 4 hours treatment. The MF of the concurrent solvent control in the third experiment was **32 x 10^{-6} without S9 mix** and 39 x 10^{-6} with S-9 mix, CE was 69-83%, SG was **27.3 fold without S-9 mix** and 42 fold with S-9 mix after 4 hours treatment. The fulfilment of acceptance criteria of the positive control could not be confirmed, as in the experiments the size of colonies were not reported. The IMF for the positive control **without S-9 mix** was **below 300 x 10^{-6}** in the second experiments, and above 300 x 10^{-6} in the third experiment (+/- S9) and in the second experiment with S-9 mix. Values in bold do not fulfil the acceptability criteria. The IMF of the treated cultures were compared to Global Evaluation Factor (GEF) from the OECD 490 (2016), 90x10^{-6} which was not exceeded for the treated cultures in all experiments. There was also no concentration related response.

The second experiment (treatment without S-9 mix) is not considered acceptable, as MF (<35 x 10^{-6}) of the negative control and IMF for the positive control (<300 x 10^{-6}) do not meet the acceptability criteria and the highest used dose (1539 µg/mL) does not show enough cytotoxicity (RTG 43.5%). The treatment with S-9 mix is considered acceptable. The variations mentioned in the third experiment are not considered to impact the study outcome, thus the study is considered acceptable.

GA4/7 is not considered clearly negative as not all acceptability criteria according to OECD 490 were fulfilled.

Table 6.4.1.3/02-1 Summary of results of Experiment 2 (without S-9 mix)

TABLE 2

MUTATION ASSAY WITHOUT ACTIVATION - TRIAL 2

A. TEST ARTICLE: GIBBERELLIC ACID A,A,
B. GENETICS ASSAY NO: 15393
C. VEHICLE: ETHANOL
D. SELECTIVE AGENT: 3.0 µg/ml TFT
E. TEST DATE: 12/28/93

TEST CONDITION:	DAILY CELL COUNTS (CELLS/ML,10E5 UNITS) 1	2	SUSPENSION GROWTH[a]		TOTAL MUTANT COLONIES	TOTAL VIABLE COLONIES	CLONING EFFICIENCY[b]		RELATIVE GROWTH (%)[c]	MUTANT FREQUENCY (10E-6 UNITS)[d]
NONACTIVATION CONTROLS[e]			AVG VEHICLE CONTROL				AVG VEHICLE CONTROL			
VEHICLE CONTROL	22.5	14.1	35.3		91	511	85.2		100.0	35.6
VEHICLE CONTROL	19.2	14.1	30.1		62	544	90.7		100.0	22.8
VEHICLE CONTROL	24.2	13.9	37.4	34.3	70	551*	91.8	89.2	100.0	25.4
MMS 10 nl/ml	12.3	11.6	15.9		332	296	49.3		25.6	224.3[f]
MMS 15 nl/ml	12.2	9.7	13.1		275	186	31.0		13.3	295.7[f]
TEST COMPOUND			RELATIVE TO VEHICLE CONTROL (%)				RELATIVE TO VEHICLE CONTROL (%)			
218 µg/ml	20.2	15.1	98.8		92	477	89.1		88.0	38.6
437 µg/ml	23.9	14.0	108.4		74	523	97.7		105.9	28.3
873 µg/ml	16.9	14.2	77.7		71	535	100.0		77.7	26.5
1310 µg/ml	16.1	12.9	67.3		79	513	95.9		64.5	30.8
1530 µg/ml	11.4	14.5	53.5		93	433	80.9		43.3	43.0

[a]SUSPENSION GROWTH = (DAY 1 COUNT/3) * (DAY 2 COUNT)/(3 OR DAY 1 COUNT IF NOT SPLIT BACK)

[b]CLONING EFFICIENCY = TOTAL VIABLE COLONY COUNT/NUMBER OF CELLS SEEDED * 100

[c]RELATIVE GROWTH = (RELATIVE SUSPENSION GROWTH * RELATIVE CLONING EFFICIENCY) / 100

[d]MUTANT FREQUENCY = (TOTAL MUTANT COLONIES/TOTAL VIABLE COLONIES) X 2X10E-4. DECIMAL IS MOVED TO EXPRESS THE FREQUENCY IN UNITS OF 10E-6

[e]VEHICLE CONTROL = 1% ETHANOL; MMS = METHYL METHANESULFONATE POSITIVE CONTROL

[f]MUTAGENIC, EXCEEDS MINIMUM CRITERION OF 55.9 X 10E-6

*ONE PLATE LOST; VALUE DETERMINED BY AVERAGING

AVG = AVERAGE

Table 6.4.1.3/02-2 Summary of results of Experiment 3 (without S-9 mix)

TABLE 3

MUTATION ASSAY WITHOUT ACTIVATION - TRIAL 3

A. TEST ARTICLE: GIBBERELLIC ACID A4A7
B. GENETICS ASSAY NO: 15393
C. VEHICLE: ETHANOL
D. SELECTIVE AGENT: 3.0 µg/ml TFT
E. TEST DATE: 01/24/94

TEST CONDITION:	DAILY CELL COUNTS (CELLS/ML,10E5 UNITS) 1	2	SUSPENSION GROWTH[a]		TOTAL MUTANT COLONIES	TOTAL VIABLE COLONIES	CLONING EFFICIENCY[b]		RELATIVE GROWTH (%)[c]	MUTANT FREQUENCY (10E-6 UNITS)[d]
NONACTIVATION CONTROLS[e]			AVG VEHICLE CONTROL				AVG VEHICLE CONTROL			
VEHICLE CONTROL	15.4	13.5	23.1		86	530	88.3		100.0	32.5
VEHICLE CONTROL	17.1	14.6	27.7		88	491	81.8		100.0	35.8
VEHICLE CONTROL	17.4	16.1	31.1	27.3	72	482	80.3	83.5	100.0	29.9
MMS 10 nl/ml	9.7	12.8	13.8		566	219	36.5		22.1	516.9[f]
MMS 15 nl/ml	9.1	10.5	10.6		356	126	21.0		9.8	565.1[f]
TEST COMPOUND			RELATIVE TO VEHICLE CONTROL (%)				RELATIVE TO VEHICLE CONTROL (%)			
437 µg/ml	14.1	11.0	63.1		95	646	128.9		81.3	29.4
873 µg/ml	10.9	14.8	65.7		72	472	94.2		61.9	30.5
1220 µg/ml	11.6	10.2	48.2		115	545	108.8		52.4	42.2
1400 µg/ml	6.8	6.6	18.3		94	450	89.8		16.4	41.8
1570 µg/ml	5.6	7.8	17.8		89	465	92.8		16.5	38.3

[a]SUSPENSION GROWTH = (DAY 1 COUNT/3) * (DAY 2 COUNT)/(3 OR DAY 1 COUNT IF NOT SPLIT BACK)

[b]CLONING EFFICIENCY = TOTAL VIABLE COLONY COUNT/NUMBER OF CELLS SEEDED * 100

[c]RELATIVE GROWTH = (RELATIVE SUSPENSION GROWTH * RELATIVE CLONING EFFICIENCY) / 100

[d]MUTANT FREQUENCY = (TOTAL MUTANT COLONIES/TOTAL VIABLE COLONIES) X 2X10E-4. DECIMAL IS MOVED TO EXPRESS THE FREQUENCY IN UNITS OF 10E-6

[e]VEHICLE CONTROL = 1% ETHANOL; MMS = METHYL METHANESULFONATE POSITIVE CONTROL

[f]MUTAGENIC, EXCEEDS MINIMUM CRITERION OF 65.5 X 10E-6

AVG = AVERAGE

Table 6.4.1.3/02-3 Summary of results of Experiment 2 (with S-9 mix)

TABLE 4

MUTATION ASSAY WITH ACTIVATION - TRIAL 2

A. TEST ARTICLE: GIBBERELLIC ACID A,A,
B. GENETICS ASSAY NO: 15393
C. VEHICLE: ETHANOL
D. SELECTIVE AGENT: 3.0 µg/ml TFT
E. TEST DATE: 12/28/93

TEST CONDITION:	DAILY CELL COUNTS (CELLS/ML,10E5 UNITS) 1	2	SUSPENSION GROWTH[a]		TOTAL MUTANT COLONIES	TOTAL VIABLE COLONIES	CLONING EFFICIENCY[b]		RELATIVE GROWTH (%)[c]	MUTANT FREQUENCY (10E-6 UNITS)[d]
S9 ACTIVATION CONTROLS[e]	S9 BATCH NO: 0473		AVG VEHICLE CONTROL						AVG VEHICLE CONTROL	
VEHICLE CONTROL	13.9	15.3	23.6		98	540	90.0		100.0	36.3
VEHICLE CONTROL	20.7	11.0	25.3		111	609	101.5		100.0	36.5
VEHICLE CONTROL	17.2	15.2	29.0	26.0	91	528	88.0	93.2	100.0	34.5
MCA 2 µg/ml	11.2	11.0	13.7		615	390	65.0		36.7	315.4[f]
MCA 4 µg/ml	13.8	8.1	12.4		600	382	63.7		32.6	314.1[f]
TEST COMPOUND			RELATIVE TO VEHICLE CONTROL (%)				RELATIVE TO VEHICLE CONTROL (%)			
218 µg/ml	11.0	13.9	65.3		118	523	93.5		61.1	45.1
437 µg/ml	13.3	13.7	77.9		125	520	93.0		72.4	48.1
873 µg/ml	5.7	13.6	33.1		138	638	114.1		37.8	43.3
1310 µg/ml	8.5	7.5	27.2		120	525	93.9		25.5	45.7
1530 µg/ml	4.7	8.3	16.7		129	387	69.2		11.6	66.7

[a]SUSPENSION GROWTH = (DAY 1 COUNT/3) * (DAY 2 COUNT)/(3 OR DAY 1 COUNT IF NOT SPLIT BACK)

[b]CLONING EFFICIENCY = TOTAL VIABLE COLONY COUNT/NUMBER OF CELLS SEEDED * 100

[c]RELATIVE GROWTH = (RELATIVE SUSPENSION GROWTH * RELATIVE CLONING EFFICIENCY) / 100

[d]MUTANT FREQUENCY = (TOTAL MUTANT COLONIES/TOTAL VIABLE COLONIES) X 2X10E-4. DECIMAL IS MOVED TO EXPRESS THE FREQUENCY IN UNITS OF 10E-6

[e]VEHICLE CONTROL = 1% ETHANOL; MCA = METHYLCHOLANTHRENE POSITIVE CONTROL

[f]MUTAGENIC, EXCEEDS MINIMUM CRITERION OF 71.5 X 10E-6

AVG = AVERAGE

Table 6.4.1.3/02-4 Summary of results of Experiment 3 (withS-9 mix)

TABLE 5

MUTATION ASSAY WITH ACTIVATION - TRIAL 3

A. TEST ARTICLE: GIBBERELLIC ACID A4A7
B. GENETICS ASSAY NO: 15393
C. VEHICLE: ETHANOL
D. SELECTIVE AGENT: 3.0 µg/ml TFT
E. TEST DATE: 01/24/94

TEST CONDITION:	DAILY CELL COUNTS (CELLS/ML,10E5 UNITS) 1	2	SUSPENSION GROWTH[a]		TOTAL MUTANT COLONIES	TOTAL VIABLE COLONIES	CLONING EFFICIENCY[b]		RELATIVE GROWTH (%)[c]	MUTANT FREQUENCY (10E-6 UNITS)[d]
S9 ACTIVATION CONTROLS[e]	S9 BATCH NO: 0473		AVG VEHICLE CONTROL				AVG VEHICLE CONTROL			
VEHICLE CONTROL	17.3	25.0	48.1		72	403	67.2		100.0	35.7
VEHICLE CONTROL	16.1	22.8	40.8		82	385	64.2		100.0	42.6
VEHICLE CONTROL	15.4	22.5	38.5	42.5	89	462	77.0	69.5	100.0	38.5
MCA 2 µg/ml	7.9	13.5	11.9		563	231	38.5		15.5	487.4[f]
MCA 4 µg/ml	4.4	9.5	4.6		521	205	34.2		5.3	508.3[f]
TEST COMPOUND			RELATIVE TO VEHICLE CONTROL (%)				RELATIVE TO VEHICLE CONTROL (%)			
437 µg/ml	11.2	15.3	44.8		91	423	101.4		45.4	43.0
873 µg/ml	11.3	13.4	39.6		113	455	109.1		43.2	49.7
1050 µg/ml	8.2	14.9	31.9		107	406	97.4		31.1	52.7
1130 µg/ml	9.5	14.1	35.0		113	441	105.8		37.0	51.2
1220 µg/ml	6.0	9.9	15.5		118	432	103.6		16.1	54.6
1310 µg/ml	4.4	8.5	9.8		136	375	89.9		8.8	72.5

[a]SUSPENSION GROWTH = (DAY 1 COUNT/3) * (DAY 2 COUNT)/(3 OR DAY 1 COUNT IF NOT SPLIT BACK)

[b]CLONING EFFICIENCY = TOTAL VIABLE COLONY COUNT/NUMBER OF CELLS SEEDED * 100

[c]RELATIVE GROWTH = (RELATIVE SUSPENSION GROWTH * RELATIVE CLONING EFFICIENCY) / 100

[d]MUTANT FREQUENCY = (TOTAL MUTANT COLONIES/TOTAL VIABLE COLONIES) X 2X10E-4. DECIMAL IS MOVED TO EXPRESS THE FREQUENCY IN UNITS OF 10E-6 .

[e]VEHICLE CONTROL = 1% ETHANOL; MCA = METHYLCHOLANTHRENE POSITIVE CONTROL

[f]MUTAGENIC, EXCEEDS MINIMUM CRITERION OF 77.9 X 10E-6

AVG = AVERAGE

B.6.4.1.3/03 Unscheduled DNA synthesis (UDS)

PREVIOUS EVALUATION	This study was evaluated in the DAR and has been considered by EFSA.
Data point addressed:	CA 5.4.1/07 (B.6.4.1.4 DAR)
Author(s) (year):	Curren R.D. (1988)
Title:	Unscheduled DNA Synthesis Assay in Rat Primary Hepatocytes with a Confirmatory Assay
Laboratory report / project number:	T8201.380009

Testing facility:	Microbiological Associates, Inc., 9900 Blackwell Road, Rockville, Maryland 20850
Published:	No
Test guideline used:	No guideline followed; in-house protocol similar to OECD 482 (now deleted)
Deviations:	The methodology is comparable to now deleted OECD 482; this assay is not required by Regulation 283/2013 however validity was confirmed with appropriate controls and the test substance was non-mutagenic in two independent experiments therefore it is included as supporting information
GLP:	Yes
EU Agreed Endpoint:	Not mutagenic

Executive summary

An *in vitro* unscheduled DNA synthesis assay was conducted with primary rat hepatocytes and gibberellins GA4/7 (batch 16-213-CD). A preliminary cytotoxicity assay was performed to determine dose levels for the main study. Five dose levels were tested in the main study, followed by a confirmatory assay. None of the test substance concentrations in either of the two completed UDS assays resulted in a significant increase in mean net nuclear silver grain counts. The positive control, DMBA, induced a significant response at both concentrations and the response in solvent and negative controls were appropriate for a valid test.

I Materials and Methods

A. Materials:

1. Test Material Gibberellins GA4/7

Description: White powder

Lot/Batch: 16-213-CD

Purity: Not stated

Stability: Assumed to be stable for the duration of the study

2. Solvent Methanol

3. Controls

Solvent/final concentration: Methanol

Positive: 7,12-dimethylbenz(a) anthracene (DMBA)

4. Test cells Hepatocytes were obtained from an adult male Fischer 344 rat and cultures were established in Williams' Medium E supplemented with 5% foetal bovine serum, L-glutamine, dexamethasone, penicillin, streptomycin and gentamicin.

B. Study Design and Methods

1. Preliminary assay

Gibberellins GA4/7 was tested in a range-finding assay at nine concentrations between 0.5 and 1500 µg/mL.

2. UDS assay

In the main study, five concentrations were fully evaluated (0.5, 1.5, 5.0, 15 and 50 µg/mL); with cytotoxicity evident at higher concentrations. A confirmatory assay was completed using an initial concentration range of 0.15-1000 µg/mL and five concentrations in the main study set at 5, 15, 50, 150 and 500 µg/mL. Both assays were evaluated for changes in the net nuclear grain count. Nuclear grains were counted in 50 cells/slide, three

slides/treatment. Net nuclear counts were obtained by subtracting cytoplasmic counts from the nuclear count. The percent cells in repair were also recorded (cells with >5 net nuclear grains).

3. Evaluation criteria

The test material was considered to show a significant result for a particular dose level if the mean net nuclear count was increased by at least five counts in comparison with controls. The test material was considered positive if at least one dose level gave a significant result and there was a dose response. In the absence of a dose response a test material may still be considered positive if two consecutive dose levels gave a significant increase in net nuclear grains. The test material was considered negative for induction of UDS if no significant increase was apparent at any dose level.

4. Statistics Not required beyond numerical comparison

II Results and Discussion

A.	Preliminary assay	The highest concentration (4000 µg/mL) resulted in formation of a precipitate when added to culture medium. Relative toxicities for concentrations of 4200, 1300 and 420 µg/mL, measured by released LDH, were 75, 48 and 39% respectively. Microscopic examination of the hepatocyte cultures indicated toxicity at doses from 42 to 4200 µg/mL. The highest concentration selected for the UDS assay was 1500 µg/mL.
B.	UDS assay	None of the test substance doses in either of the two completed UDS assays resulted in a significant increase in mean net nuclear silver grain counts. The positive control, DMBA, induced a significant response at both concentrations and the response in solvent and negative controls were appropriate for a valid test.

Conclusion

It was concluded that the test substance did not induce UDS under the conditions of this study. The endpoint was agreed in the EFSA Conclusions and is still considered valid; however the study is presented as supporting information only as investigations of potential DNA damage are not required by Regulation (EU) No 283/2013.

RMS comments and conclusion:

The study methodology is comparable to OECD 482 (1986) using male Fischer 344 rat hepatocytes and following principles of GLP. The OECD 482 was deleted in April 2014 and it is not any more required by Regulation (EU) No 283/2013. The purity was not stated, however assumed to be 90% according to the information provided in the study under B.6.2.3/02. Excessive cytotoxicity was observed in the first trial at doses from 150 µg/mL upwards. In the confirmatory trial excessive cytotoxicity was observed only at the highest dose. 1000 µg/mL. No significant induction of DNA repair synthesis was observed in the treated samples compared to negative control in both trials. Additionally, no concentration - related trend was present. Statistical analyses was not applied. Positive (DMBA) and negative controls gave the expected results.
Since OECD guidance 482 was removed and not renewed the study is considered as supportive.

b) Evaluation of additional data for the purpose of renewal of approval

No additional data was required or submitted for the purpose of renewal of approval of GA4/7.

B.6.4.2. In vivo studies in somatic cells

a) Previous evaluation (2005-2011)

B.6.4.2/01 Mammalian erythrocyte micronucleus test

PREVIOUS EVALUATION	This study was evaluated in the DAR and has been considered by EFSA.
Data point addressed:	CA 5.4.2/01 (B.6.4.1.4 DAR)
Author(s) (year):	[redacted] (1998)
Title:	GA4/7 Mouse Micronucleus Test
Laboratory report / project number:	[redacted]
Testing facility:	[redacted]
Published:	No
Test guideline used:	OECD 474, EEC Method B.12, US EPA 1997
Deviations:	The study was conducted to an earlier version of the guideline and therefore the number of cells evaluated (2000) was fewer than recommended by the current version (4000). However this is co nsidered to be a minor deviation; the study was conducted in both sexes responses were confirmed by appropriate positive controls, therefore the study is considered to be acceptable.
GLP:	Yes
EU Agreed Endpoint:	Not clastogenic

Executive summary

An *in vivo* micronucleus test was conducted with gibberellins GA4/7 (batch 287450001) in male and female CD1 mice. The test substance was administered as a single oral dose in 1% methyl cellulose at dose levels of 0 (vehicle only), 500, 1000 or 2000 mg/kg bw. Five mice/sex from each dose level were killed 24 hours following treatment; further groups of 5 mice/sex dosed with vehicle only or 2000 mg/kg bw were killed after 48 hours for bone marrow harvest. A total of 2000 erythrocytes were examined for micronuclei formation/animal. There was no biologically or statistically significant increase in the frequency of MPCEs at any dose level at either 24 or 48 hours after the treatment compared to the vehicle control. The positive control values showed large, statistically significant increases over the vehicle control values. It was concluded that gibberellins GA4/7 is not clastogenic.

I Materials and Methods

A. Materials:

1. Test Material GA4/7

Description White powder

Lot/Batch 287450001

Purity Not stated

Stability Assumed to be stable for the duration of the study

2. Vehicle 1% methyl cellulose

3. Controls

Negative 1% methyl cellulose

Positive Mitomycin C

4. Test animals Mice

Species	CD1
Age	Young adult
Weight	Not stated
Source	Not stated
Acclimation period	5-6 days
Diet	Standard Lab rodent diet (No. 1 Maintenance Diet, SQS grade obtained from [redacted]) *ad libitum*
Water	Tap water *ad libitum*
Housing	Animals were housed in a Barriered facility in standard cages

5. Environmental conditions

Temperature	19-23°C
Humidity	45-60%
Air changes	Positive pressure filtered air, number of changes/hour not specified
Photoperiod	12 hour light/dark cycle

B. Study Design and Methods

1. In life dates: 18 to 26 February 1998

2. Animal assignment and treatment

A preliminary toxicity test was conducted using dose levels of 1000 and 2000 mg/kg bw in 2 mice/sex/dose; no deaths occurred in the 48 hours post-dosing therefore 2000 mg/kg bw was selected as the highest dose for the main study. For the main study, the test substance was administered as a single oral gavage dose to groups of CD1 mice at 500 mg/kg bw (5 mice/sex), 1000 mg/kg bw (5 mice/sex) or 2000 mg/kg bw (10 mice/sex); negative controls (10 mice/sex) received the vehicle only (1% methyl cellulose; 20 mL/kg bw). The positive control was Mitomycin C (12 mg/kg bw). Five mice/sex from each dose level were killed 24 hours following treatment; further groups of 5 mice/sex dosed with vehicle only or 2000 mg/kg were killed after 48 hours.

3. Tissues and cells examined

Bone marrow smears were prepared from each animal on glass slides. A total of 2000 immature erythrocytes/animal were examined for the presence of micronuclei (micronucleated polychromatic erythrocytes; MPCEs. The proportion of immature cells was calculated for each group.

4. Details of slide preparation

Following termination both femurs were dissected and marrow cells were flushed out with 2 mL pre-filtered foetal calf serum. The recovered cells were centrifuged and resuspended in fresh serum. Single drops of the cell suspension were transferred to clean slides and left to air dry. Smears were fixed and stained prior to evaluation. The ratio of polychromatic and normochromatic erythrocytes was calculated together with appropriate group mean values and standard deviations.

5. Evaluation criteria

At least 1000 erythrocytes/animal was examined. Scoring was continued until a total of at least 2000 immature erythrocytes had been examined. A positive result was considered if a statistically significant increase in the frequency of micronucleated immature erythrocytes were observed at one or more dose levels in both sexes; the increases must be reproducible, must exceed the historical control and must show a dose-response.

6. Statistics Calculated values of micronuclei/1000 immature erythrocytes were analysed statistically using the Wilcoxon Rank Sum Test.

II Results and Discussion

A.	Clinical signs and mortality	One male mouse in the 500 mg/kg bw group exhibited hunched posture four hours after dosing, but had recovered approximately 18 hours later; no other animals displayed signs of toxicity.
B.	Micronucleus assay	The frequencies of MPCEs for the negative and positive control mice were within acceptable ranges. There was no evidence for bone marrow toxicity (depression of bone marrow proliferation) at any dose level. There was no biologically or statistically significant increase in the frequency of MPCEs at any dose level at either 24 or 48 hours after the treatment compared to the vehicle control. The positive control values showed large, statistically significant increases over the vehicle control values, demonstrating the sensitivity of the test. The ratio of polychromatic to normochromatic was in the acceptable range.

Conclusion

It was concluded that Gibberellins does not induce MPCEs in mice at doses up to the limit dose of 2000 mg/kg bw (gavage). Classification according to Regulation (EC) No 1272/2008 is not required. The *in vivo* ADME study conducted with radiolabelled gibberellins (CA 5.1.1/01) demonstrated that absorption was rapid following oral administration and radioactivity was widely distributed; it can therefore be expected that bone marrow will be exposed to the test substance following oral exposure. The endpoint was agreed in the EFSA Conclusions and is still considered valid.

RMS comments and conclusion:

The study was performed according to OECD 474 (1997) and principles of GLP. Clastogenic/aneugenic activity of GA4/7 was tested *in vivo* in CD-1 mice. The maximum dose, 2000 mg/kg, which is also the limit dose set in OECD 474 was selected based on observation in the preliminary study where 1♀ at 1000 mg/kg and 1♀ + 1♂ at 2000 mg/kg showed signs of hunched posture immediately after dosing and recovered 4 hours later. No mortality occurred.

In the main study 2000 PCE per animal were scored. There were 10 animals per treated and control group (♀5 +♂ 5) instead of 5 animals per group as stated in the current version of OECD 474, which gives 20000 PCE/group and can be considered as compliance with recent OECD 474 requirements. 1♂ at 500 mg/kg showed signs of hunched posture 4 hours after dosing and recovered 18 hours later. No blood samples were taken to investigate the level of the exposure of bone marrow to GA4/7. The ratio PCE/ total erythrocytes (PCE + NCE) was determined and for all treated groups similar to the concurrent control group values at 24- and 48-hour sampling. The ratio PCE/total erythrocyte was statistically significantly reduced in the positive control group. The HCD information provided for the negative control (see the chart below) does not contain information on the vehicle used, sampling time, type of application, number of studies performed, values for Mean±SD and do not cover the required 5 years period. However, it can be noted that the frequency of micronucleated PCEs in the 24-hour and 48 -hour concurrent negative control group were in the range of the provided HCD. HCD for the positive control were not reported, however the positive control produced a statistically significant increase in the MN/PCE compared to the control thus showing the sensitivity of the assay.

No statistically significant increase in frequency of micronucleated PCEs compared to the concurrent negative control at the 24-hour and 48-hour sampling was observed in any of the treatment groups. No dose-related increase in micronucleated PCEs was observed.

Not enough information was provided to check if all the acceptability criteria are fulfilled, thus the study result could not be considered clearly negative. The direct exposure of bone marrow is not demonstrated. According to OECD 474 only in case of intravenous administration, evidence of exposure is not needed. Direct exposure of

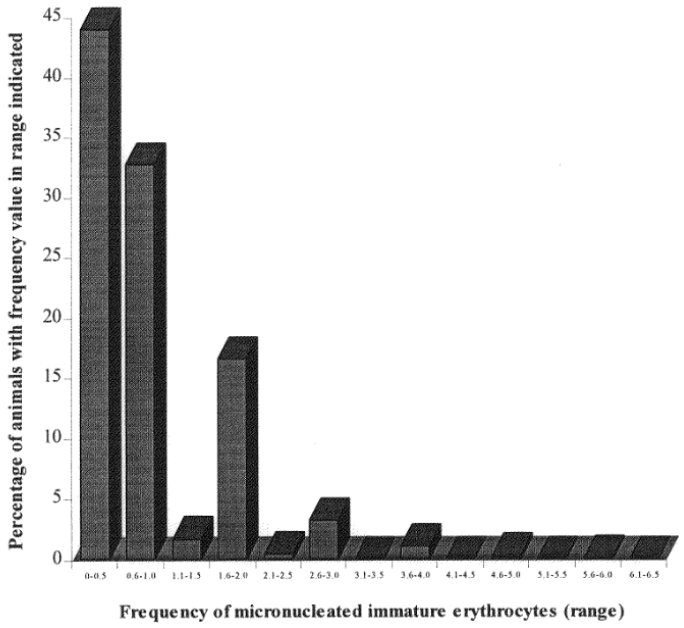
the bone marrow was observed in study B.6.4.2/02, however the test animals were mice of different strain and the dosing was intraperitoneal. Systemic exposure can be hypothesised from the observed lethargy (observed also in study under B.6.4.2/02) of which time occurrence coincides with pharmacokinetics parameters defined in rats in ADME study (B.6.1.1). Overall, there is no any other information available that could support direct exposure of the bone marrow to GA4/7 as the ADME study was done in rats and bone marrow exposure to GA4/7 was not investigated. Thus, the study is considered for supportive information.

Table B.6.4.2/01-1: Main Micronucleous test: data summary and statistical analyses

Group	Treatment (mg/kg)	Frequency of MIE cells		Statistical analysis	Prop. Imm. cells	Statistical analysis
		Mean±SD	Range			
24 hour sacrifice time						
1	1% Methyl cellulose	1.2±0.5	0.5-2.0	-	42±2	-
2	GA4/7 (500)	1.0±0.5	0.0-2.0	NS	43±3	NS
3	GA4/7 (1000)	1.1±0.7	0.0-2.5	NS	41 ±3	NS
4	GA4/7 (2000)	1.1±0.7	0.0-2.0	NS	42±2	NS
5	Mitomycin C (12)	43.7±19.8	26.3-84.4	***	39±3	*
48 hour sacrifice time						
1	1% Methyl cellulose	0.6±0.5	0.0-1.4	-	44±5	-
4	GA4/7 (2000)	0.7Ï0.4	0.0-1.5	NS	48±4	NS

Frequency of MIE cells : Frequency of micronucleated immature (polychromatic) erythrocytes
Mean±SD : Mean ± standard deviation
Prop. Imm. Cells : Proportion of Immature erythrocytes (%) (Mean ± standard deviation)
Statistical analysis : Statistical significance, compared to vehicle control values
NS Not significant, p>0.05
* Significant, p<0.05
***Very highly significant, p<0.001

Historical vehicle control data (August 1995 - December 1997)



B.6.4.2/02 Mammalian erythrocyte micronucleus test

PREVIOUS EVALUATION	This study was evaluated in the DAR and has been considered by EFSA.
Data point addressed:	CA 5.4.2/02 (B.6.4.1.4 DAR)
Author(s) (year):	[redacted] (1988)
Title:	Micronucleus Cytogenic Assay in Mice
Laboratory report / project number:	[redacted]
Testing facility:	[redacted]
Published:	No
Test guideline used:	In-house study design broadly complying with OECD TG No. 474 and EEC Method B.12
Deviations:	The dose levels are not in-line with the current guideline and only 1000 cells were scored/animal, however dose levels were selected based on the LD50 and responses were confirmed by appropriate positive controls.
GLP:	Yes
EU Agreed Endpoint:	Not clastogenic

Executive summary

The potential for gibberellins GA4/7 to increase the incidence of micronucleated polychromatic erythrocytes in bone marrow micronucleus was investigated using ICR mice. The test substance was administered as a single intraperitoneal injection at doses of 120, 600 or 1200 mg/kg bw. Marrow smears were prepared from 5 mice/sex/time point and scored for the presence of micronucleated polychromatic erythrocytes (MNPCE) and for PCE/total erythrocyte ratios. There was no increase in the number of MPCEs/1000 polychromatic erythrocytes in any of the dose groups, and therefore it was concluded that the test substance is not clastogenic.

I Materials and Methods

A. Materials:

1. Test Material Gibberellins GA4/7

Description	White powder
Lot/Batch	16-213-CD
Purity	Not stated
Stability	Assumed to be stable for the duration of the study

2. Vehicle Corn oil

3. Controls

Negative	Corn oil
Positive	Triethylenemelamine (TEM) in sterile distilled water

4. Test animals Mice

Species	ICR
Age	6-8 weeks old
Weight	29 to 37 g males; 21 to 28 g females for toxicity assay 25 to 34 g males; 20 to 29 g females for micronucleus assay
Source	[redacted]
Acclimation period	Not stated
Diet	Standard Lab rodent diet (No. 1 Maintenance Diet, SQS grade obtained from [redacted]) *ad libitum*
Water	Tap water *ad libitum*
Housing	Animals were housed in groups of up to 5 by sex in plastic cages with wire lids

5. Environmental conditions

Temperature	21-25°C
Humidity	50±20%
Air changes	Not stated
Photoperiod	12 hour light/dark cycle

B. Study Design and Methods

1. In life dates: 20 June to 24 October 1988

2. Animal assignment and treatment

The test substance was administered by single intraperitoneal injection to groups of male and female mice (5/sex/group) at doses of 120, 600 or 1200 mg/kg bw/day in the main micronucleus assay. The high dose was set at 80% of the LD50 value, following a preliminary toxicity study. The negative control group was dosed with vehicle,

corn oil, alone and the positive control groups were dosed with triethylenemelamine (TEM) at 0.5 mg/kg. Groups of mice were sacrificed at 24, 48 or 72 hours after dosing (24 hours only for positive controls).

3. Tissues and cells examined

Bone marrow smears were prepared from 5 mice/sex/time point and scored for the presence of micronucleated polychromatic erythrocytes (MPCE) and for PCE/total erythrocyte ratios. Bone marrow smears were examined for 1000 polychromatic erythrocytes (PCE)/animal. The number of normochromatic erythrocytes (NCE)/thousand PCE was recorded.

4. Details of slide preparation

Mouse bone marrow was sampled at 24, 48 and 72 hours after dosing with the test substance or the negative control, positive controls were sampled once at 24 hours post dosing. Bone marrow was collected from aspirated femurs and cell smears prepared after dispersal of marrow in foetal bovine serum followed by centrifugation. Smears were stained (May-Gruenwald and Giemsa stains) in accordance with conventional cytological procedures

5. Evaluation criteria

Two criteria were applied to determine whether the test material should be considered to show positive evidence for mutagenicity. If there were dose- and time-dependent effects consistent with a treatment-induced response and the degree of response was in relation to concurrent and historical negative and positive control data, then a statistically significant response in MN PCE frequency was considered to be treatment-related.

6. Statistics Individual animals were the units used for analysis of MN PCE frequency, PCE/total erythrocyte ratios and for bodyweight change analysis. MN PCE frequencies for each animal were transformed to the square root for analysis, PCE/total erythrocyte ratios were evaluated without transformation.

II Results and Discussion

A. Range finding test In the initial toxicity range finding assay all mice dosed at 5000, 3000 or 2000 mg/kg bw and 8/9 dosed at 4000 mg/kg bw died with 24 hours of dosing. An error in dose preparation invalidated this assay and further groups were administered doses of 781, 1093, 1531, 2143 or 3000 mg/kg bw. The majority of mice dosed at 1531 mg/kg or higher died and there was one decedent in the 1093 mg/kg group. Clinical signs observed included prostration, irregular breathing, pilo-erection, crusty eyes and lethargy and one mouse with tremors. The LD50 was calculated from the observed mortality pattern and determined by probit analysis to be approximately 1465 mg/kg bw. The high dose for the main study was set at 80% of this LD50 value.

B. Micronucleus assay All animals receiving 1200 mg/kg bw appeared lethargic approximately 2-3 hours after administration; no signs of toxicity were seen at lower dose levels. A 20-25% decrease in the ratio of polychromatic erythrocytes to total erythrocytes was evident at 24 hours after dosing in the high dose group only. Other groups were unaffected. There was no change in the number of MNPCEs/1000 polychromatic erythrocytes in any of the dose groups, at any of the sacrifice timepoints. The negative and positive controls gave the expected results.

Table B 6.4.2/02-1 Summary of results for PCE ratios in male and female mice

Harvest time (hr)	Sex	Number of animals	Mean PCE:total erythrocyte ratio				
			Vehicle control	Low dose 120 mg/kg	Mid dose 600 mg/kg	High dose 1200 mg/kg	Positive control
24	M	5	0.60	0.61	0.56	0.45	0.47

24	F	5	0.68	0.57	0.59	0.56	0.53
48	M	5	0.65	0.68	0.64	0.58	--
48	F	5	0.67	0.66	0.66	0.59	--
72	M	5	0.75	0.57	0.55	0.61	--
72	F	5	0.69	0.53	0.49	0.69	--

vehicle control: corn oil 20 mL/kg bw
positive control: TEM 0.5 mg/kg

Table B 4.4.2/02-2 Summary of micronucleus data for male and female mice

Harvest time (hr)	Sex	Number of animals	Mean micronucleated PCE/1000 PCE				
			Vehicle control	Low dose 120 mg/kg	Mid dose 600 mg/kg	High dose 1200 mg/kg	Positive control
24	M	5	0.4	1.2	0.6	1.4	49.4
24	F	5	0.4	0.6	0.4	0.2	46.0
48	M	5	0.2	1.0	0.2	0.8	--
48	F	5	0.0	0.2	0.8	0.4	--
72	M	5	0.6	0.8	1.2	0.4	--
72	F	5	0.6	0.0	0.2	0.6	--

vehicle control: corn oil 20 mL/kg bw
positive control: TEM 0.5 mg/kg

Conclusion

It was concluded that gibberellins GA4/7 is not clastogenic in this test system. Classification according to Regulation (EC) No 1272/2008 is not required. The *in vivo* ADME study conducted with radiolabelled gibberellins (CA 5.1.1/01) demonstrated that absorption was rapid following oral administration and radioactivity was widely distributed. It is also expected that bone marrow exposure to the test substance occurred due to the higher likelihood for systemic exposure following intraperitoneal exposure. In addition, a decrease in the ratio of polychromatic to total erythrocytes was evident in the high dose group, considered to be evidence of cell toxicity and therefore direct exposure to the test material. The endpoint was agreed in the EFSA Conclusions and is still considered valid.

RMS comments and conclusion:

The study was performed based on the in-house design, comparable to OECD 474 (1983) and principles of GLP. Clastogenic/aneugenic activity of GA4/7 was tested *in vivo* in ICR mice. The maximum dose was selected based on the preliminary acute intraperitoneal test where the majority of mice dosed with ≥ 1521 mg/kg died. Clinical signs and decrease in body weights were observed in groups dosed with ≥ 1521. According to the current OECD TG 474 (2016), intraperitoneal injection is not recommended, however, it was one of the recommended administration routes in studies performed in accordance with the previous versions of the guideline OECD 474 (1997 and 1983).

In the main study 1000 PCE per animal were scored. There were 10 animals per treated and control group (♀5 +♂ 5) instead of 5 animals per group as stated in the current version of OECD 474 (2016), which gives 10000 PCE/group instead of 20000 PCE/group. No blood samples were taken to investigate the level of the exposure of bone marrow to GA4/7. The ratio PCE/ total erythrocytes (PCE + NCE) was determined. A 18-25% decrease in the ratio PCE/total erythrocytes was observed in the highest dose group and 22-23% in the positive control group

when compared to the concurrent vehicle control at 24-hour sampling. All animals dosed at 1200 mg/kg appeared lethargic 2-3 hours after dosing. All other animals appeared normal. HCD for the negative control were not provided. One of the conditions for the validity of the study listen in the study report was that the mean MN/PCE must not exceed 0.5%. The concurrent control fulfilled the requirement. HCD for the positive control were not reported, however the positive control produced a statistically significant increase in the MN/PCE compared to the control thus showing the sensitivity of the assay.
No statistically significant increase in frequency of micronucleated PCEs compared to the concurrent negative control at the 24-hour, 48-hour and 72-hour sampling was observed in any of the treatment groups. No dose-related increase in micronucleated PCEs was observed.

The direct exposure of bone marrow at 24 hours was demonstrated by the decrease in the ratio PCE/total erythrocytes at the highest dose (1200 mg/kg). Systemic exposure can be also hypothesised from the time occurrence of observed clinical signs (lethargy) which coincides with pharmacokinetics parameters defined in rats in ADME study (B6.1.1). However, only half number of PCE/dose were scored and not enough information was provided to check if all the acceptability criteria of OECD 474 (2016) are fulfilled, thus the study result could not be considered clearly negative. The study is considered acceptable with limitations.

b) Evaluation of additional data for the purpose of renewal of approval

No additional data was required or submitted for the purpose of renewal of approval of GA4/7.

B.6.4.3. In vivo studies in germ cells

In vivo studies in germ cells are required when results from *in vivo* somatic cells studies are positive. GA4/7 did not show positive results in *in vivo* study on somatic cells thus, the test in germ cells is not required.

B.6.5. LONG-TERM TOXICITY AND CARCINOGENESIS

a) Previous evaluation (2005-2011)

Data on the carcinogenic potential of GA4/7 are not available and no new studies are submitted. Older published data on the carcinogenic potential of gibberellic acid GA3 in the mouse were submitted during the EU review and are available in the EU DAR, however the argument supporting read across between GA4/7 and GA3 was not considered sufficient. EFSA (2012) concluded that no further data on carcinogenicity were required. GA4/7 is a normal component of a healthy diet due to its presence in fruit and vegetables; diets high in fruit and vegetables are associated with better health therefore it is reasonable to assume that long-term exposure to gibberellins is unlikely to be harmful. Widespread natural occurrence of gibberellins in plants, fungi and bacteria has been demonstrated in a published review (MacMillan, 2002; CA 8.2.2/01): 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. Exposure to GA4/7 as a result of its use as a plant protection product is expected to be significantly lower than exposure arising from dietary consumption of fruit and vegetables. In addition, the available studies do not indicate any increased risk associated with long-term exposure to GA4/7; the substance is not acutely toxic, is not genotoxic, and does not impair development or reproduction.

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

No new studies were submitted for the purpose of renewal of Annex I inclusion.

RMS comments and conclusion:

Additional submission of studies on carcinogenesis was not considered necessary as the missing information of the provided toxicology database was taken into account in the setting of reference values. The observed effects in short term studies at the LOAEL in the rat and dog study are relatively marginal, residue levels due to use of GA4/7 in PPP are similar to the natural levels in plants, the *in vivo* genotoxicity studies point more on the result of being non-genotoxic and no accumulation is to be expected based on ADME studies.

For information purposes taken from Vol 3, B7, Point B.7.3.:
GA_4 has been identified in 54 plant species, 7 fungi and 3 bacteria species. In plants GA_4 was found mainly in seeds, leaves, shoots, buds, fruits and pollen. GA_7 has been identified in 14 plant species and 1 fungus species. In plants GA_7 was found mainly in seeds, leaves, shoots and pollen.
Untreated apples: In scientific papers natural background concentration of GA_4 in apples was up to 0.00017 mg/kg and natural background concentration of GA_7 up to 0.000004 mg/kg. We can conclude that the use of $GA_{4/7}$ as a plant protection product results in residue levels similar to the natural levels in plants.

Untreated pears: In scientific papers natural background concentration of GAs in pears was up to 0.06 mg/kg. We can conclude that the use of $GA_{4/7}$ as a plant protection product results in residue levels similar to the natural levels in plants.

B.6.6. REPRODUCTIVE TOXICITY

B.6.6.1. Generational studies

Data on reproductive toxicity over two generations in the rat were submitted during the EU review of GA4/7 and are available in the EU DAR. The study was considered acceptable in the EFSA conclusion and is considered adequate for supporting renewal of GA4/7; no new reproductive toxicity studies are submitted. EFSA (2012) concluded that fertility and overall reproductive performance was not impaired following GA4/7 exposure. The parental NOAEL was 300 mg/kg bw/d; this value was used to derive the AOEL.

PREVIOUS EVALUATION	This study was evaluated in the DAR and has been considered by EFSA.
Data point addressed:	CA 5.6.1/01 (B.6.6.1 DAR)
Author(s) (year):	[redacted] (2001)
Title:	A Dietary Two-Generation Reproductive Toxicity Study of Gibberellin (GA4A7) in Rats
Laboratory report / project number:	[redacted]
Testing facility:	[redacted]
Published:	No
Test guideline used:	OECD 416, USEPA OPPTS 870.3800
Deviations:	No major deviations; the study is acceptable and was used for AOEL derivation (EFSA, 2012).
GLP:	Yes
EU Agreed Endpoint:	Parental NOAEL: 300 mg/kg bw/d (females)

	Offspring NOAEL: 600 mg/kg bw/d Reproductive NOAEL: 1000 mg/kg bw/d

Executive summary

Groups of 30 male and 30 female rats were administered gibberellins GA4/7 (purity 90.8%, batch no 33263CD00) in the diet at dose levels of 0, 300, 600 or 1000 mg/kg bw/d over two generations. There were no treatment-related deaths during the study, and no clinical signs considered to be treatment-related. Parental toxicity as evidenced by reductions in body weight was observed in the F0 generation (both sexes) at 1000 mg/kg bw/d and in the F1 generation at 600 mg/kg bw/d for males, and at 1000 mg/kg bw/d for both sexes. In both generations, food consumption evaluated as g/kg/day, was increased in the 1000 mg/kg bw/d dose group. Because increases in food consumption were not accompanied by gains in body weight, the reductions in body weight gain were considered to be treatment-related. Dose-related increases in the incidences and severity of nephropathy, medullary tubular dilatation, medullary fibroplasia and medullary basophilic interstitium were observed in the 600 and 1000 mg/kg bw/d F0 females. These renal findings, as well as medullary tubule hyperplasia were also observed in a dose-related manner in the 600 and 1000 mg/kg bw/d F1 females. Neonatal toxicity was observed in the F1 and F2 litters as a reduction in mean pup body weight, body weight gain and spleen weight in the 1000 mg/kg bw/d group. Mean thymus weight was reduced in the 1000 mg/kg bw/d F2 pups. Fertility and overall reproductive performance were not impaired at any dose level.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material Gibberellins GA4/7

Description	Fine white powder
Lot/Batch	33263CD
Purity	72.5%GA4 90.8%GA4/7 Test material dietary concentrations were corrected to 100%, using a factor of 1.1, to adjust for test article purity
Stability	Analysis of dose formulations indicated material was stable in diet, at room temperature and under frozen conditions for at least 15 days. Homogeneity tests indicated that gibberellins GA4/7 was uniformly dispersed in rodent diet.

2. Vehicle Diet

3. Test animals

Species	Rat
Strain	Sprague Dawley Crl:CD(SD)IGS BR
Age	Young adult (38 days old)
Source	[redacted]
Weight	196-283 g F0 males; 147-210 g F0 females. (Protocol specified minimum weight was 200 g for males and 150 g for females)
Acclimation time	14 days

Diet	Standard Lab rodent diet (Purina Certified Rodent Lab Diet 5002) *ad libitum*
Water	Reverse osmosis purified water *ad libitum*
Housing	Animals were individually housed in suspended stainless steel cages. During the period of cohabitation, 1 male and 1 female were paired in a suspended wire mesh cage. Successfully mated females were transferred to plastic cages, in which they remained throughout lactation and were then returned to suspended stainless steel cages after weaning.

4. Environmental conditions

Temperature	20.1-22.8°C
Humidity	25-64%
Air changes	Not stated
Photoperiod	12 hour light/dark cycle

B. Study Design and Methods

1. In life dates: 23 November 1998 to 5 October 1999.

2. Animal assignment and treatment

Thirty male and thirty female rats were allocated to four groups of the study as the F0 generation. The rats were dosed with gibberellins GA4/7 for ten weeks at dietary concentrations prepared to achieve doses of 0, 300, 600 or 1000 mg/kg bw/d. Following ten weeks of treatment the rats were paired 1:1 and mated to assess reproductive performance. From the resultant (weaned) litters 30 F_1 pups/sex/group were selected for the F1 generation. The F0 parents and non-selected F_1 weanlings were sacrificed at this point and subjected to pathological evaluation. The selected F1 animals were treated with test diet at dose levels of 0, 300, 600 or 1000 mg/kg bw/d for ten weeks and then the males were mated 1:1 with a female of the same treatment group (avoiding sibling matings) to assess reproductive performance. On day 21 post-partum, the F1 parental rats and F_2 weanlings were terminated and subjected to pathological evaluation.

Test and control diet was supplied *ad libitum* to the F0 parents for 70 days prior to mating and then throughout the periods of gestation and lactation up to time of necropsy. The F1 offspring, potentially exposed to the test substance *in utero*, were supplied with treated diet from one day after weaning (postnatal day 22) through to necropsy. The F2 offspring were exposed, potentially *in utero* and during nursing up to post-natal day 21. Test material concentration in diet was set based upon the food consumption and body weight data from the last week of the mating period. Initial test diets for the F1 weanlings were based on a bodyweight of 50 g and food consumption value of 15 g/rat/day.

3. Dose preparation

Weighed test material for each dose level was mixed with 1 kg diet to produce the pre-mix. The premix was then dispersed into a suitable size batch of basal diet to prepare sufficient test diet for one week's administration. Prepared diets were stored at room temperature until use. Diets were analysed for achieved concentration, stability and homogeneity prior to dosing and at weekly intervals for the first month of the study and then once a month for the remainder of the study.

4. In-life observations

All animals were observed twice daily for mortality/moribundity, appearance, behaviour and pharmacotoxic signs. A detailed clinical examination was completed at weekly intervals for all parents. Females were also checked twice daily during expected and actual parturition for dystocia or other difficulties. Male bodyweights were recorded weekly from one week prior to commencing treatment (F0) to the scheduled necropsy of the F1 parents. Female weights were recorded at weekly intervals from one week prior to dosing (F0) to observation of copulation and then on gestation days 0, 4, 7, 11, 14 and 20 and through lactation on days 1, 4, 7, 14 and 21. Females were weighed weekly from after weaning to necropsy. Food consumption was measured at weekly intervals from one week prior to dosing up to time of pairing in each generation. Male food consumption was subsequently measured at weekly intervals while female consumption was recorded on gestation days 0, 4, 7, 11, 14 and 20 and through lactation on days 1, 4, 7, 14 and 21. Food efficiency percent was calculated from the weight change/interval divided by food consumed/individual in the interval x 100. Mean amount test substance consumed was calculated from the dietary concentration of test material and the amount of food consumed.

5. Reproductive parameters and litter data

Daily vaginal smears were prepared and examined (from 21 days prior to pairing up to observation of copulation) for each female to determine the average cycle length for complete cycles, calculated as the number of returns to metoestrous (M) or dioestrous (D) from oestrous (E) or pro-oestrous (P). Cycle length was the number of days from M or D to next M or D. Rats were paired 1:1 within treatment groups for up to 15 days and examined daily for evidence of mating. Positive evidence of mating taken to be a copulatory plug or sperm in vaginal smear; day 0 of gestation was identified as day positive evidence was observed. The pair was separated after positive evidence of mating was found. F0 parents were mated once to produce a single litter. The animals were approximately 17 weeks old at time of pairing. The same procedures were followed for animals randomly selected as F1 parents (avoiding sibling matings). These rats were approximately 14 to 16 weeks at time of pairing.

For the F0 and F1 generations all females were allowed to deliver litters and rear young to weaning (post-natal day 21). On the day parturition was completed, the duration of gestation was calculated, litters were sexed and examined for gross malformations and numbers of live and stillborn pups counted.

Each litter (F1 and F2 offspring) was examined twice daily for survival and a daily record of litter size was maintained. Any pups dying before post-natal day 4 were subject to necropsy and any pups with external abnormalities were cleared and stained for skeletal examination and any developmental variations or malformations were recorded. Litters were culled on postnatal day 4 to eight pups/litter, 4 males and 4 females, wherever possible, to reduce variability. Pups dying after postnatal day 4 but before weaning were necropsied and tissues indicated by gross abnormalities were saved for histopathology. A daily clinical record of changes in appearance or behaviour was maintained and pups were subject to a detailed physical examination on post-natal days 1, 4, 7, 14 and 21, when they were also weighed. Determination of pup sex was completed on days 0, 4, 7, 14 and 21. Various litter parameters and indices were calculated during the lactation phase. Litters were weaned on lactation day 21 and 30 male and 30 female pups were selected from each treatment group to comprise the F1 parental generation, which were treated with test diet from day 22 *post-partum*. Maturation of selected F1 pups, 30/group, was assessed by observing for balanopreputial separation in males (checked from post-natal day 35) or for vaginal perforation in females (checked from post-natal day 35).

For all F0 and F1 males the right epididymis was excised during and weighed immediately after death. The right cauda epididymis was incised and placed in Dulbecco's phosphate buffered saline with Bovine Serum Albumin for incubation for 10 minutes. Sperm motility, expressed as percent motile, was determined by counting 200 motile and non-motile spermatozoa under constant temperature using a Hamilton-Thorne computer-assisted sperm analysis system. Sperm morphology was evaluated for 200 spermatozoa/rat following techniques described by Linder *et al*. The left testis and epididymis from all F0 and F1 males were frozen, homogenised and evaluated for homogenisation-resistant spermatids and the sperm production rate.

6. Post mortem observations

F0 surviving parents were all killed following selection of the F1 generation and subjected to gross pathological examination during necropsy. The F1 parents were killed after weaning of the F2 pups and were similarly subject to necropsy. The F1 and F2 pups were killed on post-natal day 21 and subject to necropsy, except for the 30 F1 pups/sex/group selected to be the F1 parental generation and one pup/sex litter from each of the F1 and F2 weanlings selected for complete necropsy on post-natal day 21. Necropsy involved examination of all external surfaces, all orifices, cranial cavity, external surfaces of brain and spinal cord and the thoracic, abdominal and pelvic cavities with their viscera. A full EC compliant list of tissues was preserved for each animal and microscopic investigations completed for 10 F0 and F1 animals/sex/group. Absolute and relative (organ to bodyweight ratios) organ weights were recorded, including adrenals, brain, epididymides, kidneys, liver, ovaries, pituitary, prostate, seminal vesicles, spleen, testes, thymus and uterus with oviducts and cervix.

5. Statistics Analyses were conducted using Chi-square test with Yates' correction factor, one-way ANOVA with Dunnett's test, Kruskal-Wallis test with Mann-Whitney U test, Kolmogorov-Smirnov test.

II Results and Discussion

A. Clinical signs and mortality

F0 generation: There were no unscheduled deaths during the study. None of the infrequently occurring clinical signs (soft stools, red material around nose/eyes, hair loss, scabbed body surfaces) showed any dose-relationship and commonly occurred at similar frequency in the control group. None of the findings were considered to be treatment-related.

F1 generation: One high dose group (1000 mg/kg bw/d) male was terminated *in extremis* during week 26. Abdominal swelling, observed for the previous 4 weeks, was revealed as a large ventral mass during necropsy and confirmed as rhabdomyosarcoma and as such was unrelated to treatment. All other F1 parents survived to scheduled termination. Infre

quently observed signs were either also observed in controls or were not present in any dose-related pattern.

B. Body weight and food consumption

Mean body weights and body weight gains were reduced for both males and females in the 1000 mg/kg bw/d group throughout the majority of the F0 and F1 generations. In both generations, food consumption was increased in the 1000 mg/kg bw/d group. Because increase in food consumption were not accompanied by gains in body weight, the reductions in body weight were considered to be treatment related. Mean body weights were also reduced for F1 males in the 300 and 600 mg/kg bw/d groups. Reductions were observed in the 300 mg/kg bw/d group beginning at week 34 and in the 600 mg/kg bw/d group beginning at week 25. The reductions were not considered treatment related in the 300 mg/kg bw/d group due to later onset and lack of other signs of toxicity.

C. Reproductive parameters

F0 generation: There was no effect on reproductive performance in any of the treated groups. There were no significant differences from controls. The numbers of non-pregnant females and the number of males failing to sire a litter were 2, 3, 4 and 1 in the control, 300, 600 and 1000 mg/kg bw/d groups. None of the females that delivered had a total litter loss between lactation day 0 and 21. There were no treatment-related effects on mean period between pairing and coitus and no significant differences between treated and control groups. Mean oestrous cycle length was 4.5; 4.6; 4.2 and 4.6 days for control, 300, 600 and 1000 mg/kg bw/d groups. There was no treatment-related disruption of the oestrous cycle. The mean numbers of liveborn pups/litter for the respective control and treated groups were 13.2; 12.4; 13.5 and 13.6. Mean gestation length for females dosed at 300, 600 or 1000 mg/kg bw/d was 21.8, 21.8 and 21.9 days. These values were similar to the concurrent control, 22.1 days and historical control values (21.9 days). There were no signs of dystocia.

There were no effects of treatment on mean testicular and epididymal sperm numbers; on sperm production rates;

sperm motility or the percentage with normal morphology.
F1 generation: There was no effect on reproductive performance in any of the treated groups. There were no significant differences from controls. The numbers of non-pregnant females in the control, 300, 600 and 1000 mg/kg bw/d groups were 2, 3, 0, 0 respectively. The number of males failing to sire a litter were 2, 4, 1 and 0 in the control, 300, 600 and 1000 mg/kg bw/d groups. None of the females that delivered had a total litter loss between lactation day 0 and 21. There were no treatment-related effects on the mean length of the period between pairing and coitus and no significant differences between treated and control groups. Mean oestrous cycle length was 4.4; 4.8; 4.4 and 5.3 days for control, 300, 600 and 1000 mg/kg bw/d groups. There was no treatment-related disruption of the oestrous cycle. The mean numbers of liveborn pups/litter for the respective control and treated groups were 13.3; 13.2; 13.2 and 13.0. Mean gestation length for females dosed at 300, 600 or 1000 mg/kg bw/d was 22.0, 21.9 and 21.8 days. These values were similar to the concurrent control, 21.8 days and historical control values (21.9 days). There were no signs of dystocia.
There were no effects of treatment on mean testicular and epididymal sperm numbers; on sperm production rates; sperm motility or the percentage with normal morphology.

D. Post mortem findings

Increased incidence and severity of renal macroscopic and microscopic findings were observed for the 600 and 1000 mg/kg bw/d group F0 and F1 females (nephropathy and medullary tubule dilatation, fibroplasia and interstitial basophilia). The findings were generally minimal or mild in extent. Increases in ovarian primordial follicle counts were observed in the 1000 mg/kg bw/d group F1 females but were considered to be the result of biological variability.

E. Litter findings

F1 litters: There was no effect of treatment on live litter size, mean number of pups born or the sex ratio (% males) of litters at birth. Post-natal survival, pre- and post-selective culling was unaffected by treatment. The values for all litter parameters were within the range of background data for the conducting laboratory. There were no treatment-related effects on general physical condition of the F1 litters. In general changes in condition occurred in a non-dose related manner or occurred in single litters. The number of pups found dead or killed during the lactation phase were 7, 11, 2 or 17 for controls and 300, 600 or 1000 mg/kg bw/d groups respectively and the numbers missing, presumed cannibalised, in these groups were 2, 10, 4 and 12. The incidence of uneven hair growth and subcutaneous haemorrhage appeared greater among treated groups. A treatment-related reduction in bodyweight was evident for the high dose pups. Reduced offspring weights were recorded in this group on Days 4, 7, 14 and 21. The effects noted in the F1 pups were repeated in the F2 pups. F1 pup weight gains were significantly reduced for the high dose males and females on days 4-7 and 7-14, reflecting no recovery from the reduced bodyweights from post-natal day 1. In comparison with controls the reductions on post-natal day 1, for the treated groups 300, 600 or 1000 mg/kg bw/d, were 8.2, 8.2 or 6.8% for males and 7.2% for each of the female groups. Since these showed no dose relationship and the effect was not replicated in the F2 offspring, the bodyweight reduction at the lower dose levels was not considered an adverse effect of treatment.
F2 litters: The values for all litter parameters were similar for control and treated groups. Mean live litter size, mean number of pups born and the sex ratio (% males) at birth were all unaffected by treatment. Survival, prior to and post-selection, was also unaffected by treatment. There were no treatment-related effects on general physical condition or viability of the F2 litters. The number of pups found dead during the lactation phase were 13, 18, 8 or 11 for controls and 300, 600 or 1000 mg/kg bw/d groups respectively and the numbers missing, presumed cannibalised, in these groups were 2, 4, 5 and 2. The incidence of uneven hair growth and subcutaneous haemorrhage appeared greater among treated groups. Other clinical effects included one or three pups, from the 600 or 1000 mg/kg bw/d group respectively, gasping; one high dose pup with abnormal gait (rocking, lurching or swaying) and 23 pups in the low dose group with hair loss. Weight reductions for male and female pups were reduced on post-natal day 21 and for males only on day 14 with reduced weight gains recorded in the high dose group for males and females over the periods day 7 to 14 and 14 to 21. The effects on bodyweight were attributed to

treatment at 1000 mg/kg bw/d. Sporadic effects on bodyweight in the groups dosed at 300 or 600 mg/kg bw/d were not considered to be treatment-related.

Neonatal toxicity was evident for the high dose group, 1000 mg/kg bw/d, in both F1 and F2 litters where reduced pre-weaning body weights and lower spleen weight (F1 and F2) and reduced thymus weight (F_2) were evident. There was no evidence of neonatal treatment related effects at dose of 300 or 600 mg/kg bw/d. No evidence of reproductive toxicity was seen at any of the dose levels used in this study.

Table B 6.6.1-1 Summary of histopathological renal changes for F0 females and testicular changes in F0 males

	Males				**Females**			
Dose level (mg/kg bw/d)	**0**	**300**	**600**	**1000**	**0**	**300**	**600**	**1000**
Number of animals examined	**13**	**1**	**1**	**12**	**30**	**30**	**30**	**30**
Kidneys (individual incidence)								
Number unremarkable	Not applicable				10	8	12	0
Nephropathy - total					2	2	11	28*
minimal					2	2	8	20
mild					0	0	3	6
moderate					0	0	0	2
Subacute inflammation - minimal					8	11	5	1
Tubular mineralisation - minimal					10	10	4	4
Hydronephrosis-minimal					0	0	1	0
Cortical cysts-mild					0	0	1	0
Urothelial, medullary papilla hyperplasia					0	0	6	4
minimal					0	0	3	3
mild					0	0	3	1
Chronic active pyelonephritis					0	0	0	2
mild					0	0	0	1
moderate					0	0	0	1
Tubular epithelium medullary hyperplasia					0	0	7	13*
minimal					0	0	7	12
mild					0	0	0	1
Tubular dilatation					1	0	9	22*
minimal					1	0	9	20
mild					0	0	0	2
Interstitial medulla basophilia					0	0	8	22*
minimal					0	0	7	19
mild					0	0	1	3
Medulla subacute inflammation - minimal					0	0	4	9
Medulla fibroplasia					0	0	9	19*
minimal					0	0	9	17
mild					0	0	0	2
Medulla acute inflammation - minimal					0	0	0	1
Testes (individual incidence)								
Right epididymis-No. examined	10	na	1	11	Not applicable			
subacute inflammation-minimal	2	na	1	0				
aspermia - severe	0	na	1	1				

Vas deferens-examined /unremarkable	10	na	na	11	
Prostate - No. examined	10	na	na	11	
chronic active inflammation-minimal	1	na	na	1	
subacute inflammation - minimal	1	na	na	0	
Seminal vesicles - No. examined	10	na	na	11	
subacute inflammation - minimal	1	na	na	0	
Right testis-No. examined	30	29	30	30	
seminiferous tubule degeneration	0	0	1	2	
minimal	0	0	0	1	
moderate	0	0	1	1	
subacute inflammation - minimal	0	1	0	0	

* *Statistically significant difference from controls. p<0.05, using Kolmogorov-Smirnov one-tailed test.*

Table B 6.6.1-2 Summary of histopathological renal changes for F1 females

	Females			
Dose level (mg/kg bw/d)	**0**	**300**	**600**	**1000**
Number of animals examined	**30**	**30**	**30**	**30**
Kidneys (individual incidence)				
Number unremarkable	7	7	6	1
Nephropathy - total	8	5	13	27*
minimal	8	5	9	18
mild	0	0	4	7
moderate	0	0	0	2
Subacute cortical inflammation - minimal	8	7	8	1
Tubular mineralisation - minimal	11	6	5	3
Multilamellar body-minimal	1	0	1	0
Medullary cysts-mild	0	0	1	0
Urothelial, medullary papilla hyperplasia	0	0	7	6
minimal	0	0	6	5
mild	0	0	1	1
Tubular epithelium medullary hyperplasia	0	0	3	10*
minimal	0	0	3	9
mild	0	0	0	1
Tubular dilatation	1	0	6	20*
minimal	0	0	4	16
mild	1	0	2	4
Medulla tubular necrosis - minimal	0	1	0	1
Interstitial medulla basophilia	0	3	9	21*
minimal	0	3	4	14
mild	0	0	5	5
moderate	0	0	0	2
Medulla subacute inflammation - minimal	1	4	1	10

Medulla fibroplasia minimal mild	1 1 0	0 0 0	4 4 0	21* 20 1
Medulla acute inflammation - minimal	0	0	3	4

* *Statistically significant difference from controls. p<0.05, using Kolmogorov-Smirnov one-tailed test.*

Conclusion

Based on the above; the NOAEL for parental toxicity was considered to be 300 mg/kg bw/d; the NOAEL for neonatal toxicity was considered to be 600 mg/kg bw/d; and the NOAEL for reproductive toxicity was considered to be 1000 mg/kg bw/d (the highest dose tested). The endpoints were agreed in the EFSA Conclusions and are still considered valid.

RMS comments and conclusion:

The study follows OECD 416 and principles of GLP. The study is acceptable with limitations. Despite it was performed in 1998, the study adequately covered some new requirements of the latest version of OECD 416 (2001) with some deviations: thyroid of parenteral animals was not weighted, the weight of paired organs was not reported individually, thus a mean weigh for kidneys, adrenals, ovaries, testes, and epididymis is available. For both F0 and F1 parents the histopathology was performed on approximately 10 rats/sex/group and not on all parenteral animals selected for mating in the control and high dose group as it is required for examination of vagina, uterus with cervix, ovaries, one testis, one epididymis, seminal vesicles, prostate and coagulating gland. The exception was the histopathological investigation of right testis in the F0 generation which were examined in all the parenteral animals in the control and high dose group (30 animals/dose.)

In the study, there were no treatment related mortalities and/or clinical findings observed for both generations (F0, F1) of parenteral animals. In both generations at 1000 mg/kg bw/day, reductions in body weight gain were considered as treatment related effect due to increased food consumption (g/kg/day) with no accompanied gains in body weight. In all dose levels in both generations no treatment related effects were observed on: reproductive performance, precoital interval, regularity and duration of oestrus cycle, gestation length and spermatogenic endpoints (testicular and epididymal sperm numbers, sperm production rate, sperm motility and/or the percentage of morphologically normal sperm). Mean values for all endpoints of reproductive function fell within the ranges of historical control data of the laboratory (except the length of oestrus cycle length could not be checked as it was not reported in the HCD).

At necropsy, any absolute and relative organ weight changes observed in the F0 and F1 parental animals were considered to be incidental or related to the decreased terminal body weights of these animals, since there were no macroscopic or microscopic findings in these tissues. In the 600 and 1000 mg/kg as/day group of F0 and F1 females, an increase in incidence and severity in microscopic pathological findings in kidneys were observed and thus, considered to be treatment related. The interstitial medullar basophilia of minimal severity was found in 3/30 F1 females of the 300 mg/kg bw/day group. The finding was stated to be unilateral, focal and of low severity, thus with no other additional pathological renal findings at this dose and lack of clinical findings it was not considered an adverse effect. In F0 males, the observed diffuse seminiferous tubule degeneration was not considered treatment related as no increase in severity and no concurrent change in testicular weight occurred. Diffuse seminiferous tubule degeneration was not observed in the examined testis of 10 F1 males of the control and high dose group. In F1 females an increase in mean number of primordial follicles was observe at 1000 mg/kg bw/day (118.4) compared to control (85.59) which lacking of statistically significance was attributed to biological variability.

The mean live litter size, mean number of pups born and sex ratio at birth were unaffected by the treatment in both the F1 and F2 litters. There were no treatment-related effects on general physical condition or viability of pups in either generation. The incidence of subcutaneous haemorrhages and uneven hair growth appeared to occur more frequently in the treated litters in both generations. The effects were not considered treatment related as they occurred in single litters or did not occur in a dose-related manner. A decrease in the body weight compared to control was observed in males and females at PND 21 in the first and second generation at 1000 mg/kg bw/day. Thus, the effect was considered treatment related. There were no necropsy remarkable findings for pups found dead during the postnatal period. Mean absolute spleen weights for F1 males and females pups were significantly reduced at 1000 mg/kg bw/day when compared to the control group, 19.4% and 17.8 %, respectively. The same effect was also observed in the second generation thus, the reductions were considered to be treatment related. In the F2 generation, mean absolute and relative spleen weights were significantly reduced in males (22%) and females (25%) of the 1000 mg/kg bw/day group. Additionally, mean absolute thymus weights were reduced in F2 males (significantly, 16%) and females (11%). Necropsy of the pups showed no treatment-related findings. Landmarks of sexual maturation (balanopreputial separation and vaginal patency) in the F1 generation were not affected adversely in the treated groups. The mean values fell within the ranges of historical control data of the laboratory: balanopreputila separation (mean: 44.5, range:41.6-49.0), vaginal patency (mean: 33.2, range: 31.9-38.8).

Table B.6.6.1.-3 F0 generation findings

F0 generation and F1 litters		Dose (mg/kg bw)			
		0	300	600	1000
F0 Survival	♂	30/30	30/30	30/30	30/30
	♀	30/30	30/30	30/30	30/30
General observation		Reduced body weight, altered food consumption patterns			
F0 reproductive performance (%)					
mating indices		100	100	100	100
fertility indices		93.3	90.0	86.7	96.7
F0 mean bodyweight (g)					
♂	pre-administration	178	178	178	178
	end of pre-mating period	518	505	498	492
	end of mating	540	527	517	510
	termination	592	572	563	553*
♀	pre-administration	146	145	146	146
	start of gestation	283	275	274	268
	end of gestation	396	386	394	380
	start lactation	308	298	301	286
	end of lactation (weaning)	332	329	333	320
	termination	321	309	311	301**
F0 mean organ weight (g)					
♂	brain	2.08	2.09	2.09	2.07
	liver	19.18	18.88	19.69	19.90
	spleen	0.84	0.86	0.87	0.86
	seminal vesicles/coagulation gland	2.80	2.73	2.79	2.64
	prostate	0.65	0.70	0.68	0.67
	testis – right	1.73	1.69	1.65	1.70
	testis – left	1.75	1.68	1.66	1.70
	epididymis – right	0.71	0.70	0.70	0.70

epididymis – left	0.74	0.71	0.71	0.72
cauda epididymis – right	0.3157	0.3183	0.3174	0.3162
cauda epididymis – left	0.3280	0.3234	0.3280	0.3270
thymus	0.2452	0.2258	0.2355	0.2262
adrenals	0.0599	0.0631	0.0643	0.0639
pituitary	0.0160	0.0157	0.0150	0.0155
♀ brain	1.92	1.91	1.89	1.89
liver	12.05	11.92	12.02	11.86
kidneys	2.30	2.18	2.14	2.26
spleen	0.65	0.62	0.61	0.61
uterus/oviducts/cervix	0.70	0.68	0.71	0.62
ovaries	0.1149	0.1105	0.1018*	0.1108
thymus	0.2654	0.2907	0.2578	0.3003
adrenals	0.0736	0.0748	0.0735	0.0785
pituitary	0.0200	0.0209	0.0205	0.0189
F1 neonatal data				
mean male body weight (g) on day 1	7.3	6.7**	6.7**	6.8*
mean female body weight (g) on day 1	6.9	6.4*	6.4*	6.4**
mean male body weight (g) on day 21	50.6	48.7	51.5	46.8**
mean female body weight (g) on day 21	49.2	46.4*	48.4	44.6**
% survival, post natal day 0	98.2	98.9	99.7	97.1
% survival, post natal day 0 to 1	99.7	97.6	98.8	97.5
% survival, post natal day 1 to 4 (pre-selection)	99.7	98.0	100.0	98.4
% survival, birth to post natal day 4	97.7	94.5	98.4	93.3
% survival, post natal day 4 to 21	100.0	99.1	100.0	100.0
Mean day of acquisition for developmental landmarks:				
balanopreputial separation	44.6	45.5	45.9	46.1
vaginal patency	32.6	32.5	32.9	32.8
Organ weights (g) for selected weanlings:				
♂:				
brain	1.4267	1.4545	1.4667	1.4408
spleen	0.2414	0.2138	0.2244	0.1946**
thymus	0.2105	0.2146	0.2251	0.2111
♀:				
brain	1.3946	1.4102	1.4345	1.4027
spleen	0.2432	0.2240	0.2208	0.1998**
thymus	0.2305	0.2290	0.2388	0.2484

* *Statistically significant difference from controls. p<0.05, using Dunnett's test.*

* *Statistically significant difference from controls. p<0.01, using Dunnett's test.*

Table B.6.6.1.-4 F1 generation findings

F1 generation and F2 litters		Dose (mg/kg bw)			
		0	300	600	1000
F1 Survival	♂	30/30	30/30	30/30	29/30
	♀	30/30	30/30	30/30	30/30
General observation		Reduced body weight, altered food consumption patterns			
F1 reproductive performance (%)					
mating indices		100	96.7	96.7	100
fertility indices		93.3	86.7	96.7	100
F1 mean bodyweight (g)					
♂	pre-administration	76	74	79	66**
	end of pre-mating period	533	511	503	488**
	end of mating	584	560	551*	523*
	termination	642	607*	597**	566**
♀	pre-administration	71	66	70	61**
	start of gestation	283	281	277	264**
	end of gestation	410	400	393	379**
	start lactation	319	313	305	290**
	end of lactation (weaning)	334	341	334	318*
	termination	333	320	318	304
F1 mean organ weight (g)					
♂	brain	2.12	2.13	2.14	2.08
	liver	23.61	20.89*	22.15	20.91*
	kidneys	4.35	4.13	4.16	3.89*
	spleen	0.96	0.91	0.90	0.88
	seminal vesicles/coagulation gland	1.76	2.04	1.93	1.92
	prostate	1.18	1.28	1.26	1.29
	testis – right	1.79	1.81	1.86	1.77
	testis – left	1.81	1.84	1.85	1.76
	epididymis – right	0.73	0.72	0.73	0.70
	epididymis – left	0.76	0.76	0.76	0.73
	cauda epididymis – right	0.3210	0.3221	0.3272	0.3197
	cauda epididymis – left	0.3271	0.3431	0.3340	0.3171
	thymus	0.3170	0.2976	0.2842	0.2991
	adrenals	0.0661	0.0695	0.0709	0.0692
	pituitary	0.0181	0.0178	0.0184	0.0179
♀	brain	1.98	1.96	1.94	1.91
	liver	12.41	11.75	12.33	11.53*
	kidneys	2.41	2.25	2.30	2.28
	spleen	0.66	0.68	0.65	0.63
	uterus/oviducts/cervix	0.74	0.80	0.71	0.71
	ovaries	0.1225	0.1214	0.1102	0.1099*
	thymus	0.3119	0.3302	0.3018	0.3494
	adrenals	0.0783	0.0862	0.0817	0.0818
	pituitary	0.0223	0.0227	0.0207	0.0198
F2 neonatal data					
mean ♂ body weight (g) on day 1		7.1	6.7	6.8	6.8
mean ♀ body weight (g) on day 1		6.7	6.3	6.4	6.3
mean ♂ body weight (g) on day 21		52.1	49.7	49.2	46.9**
mean ♀ body weight (g) on day 21		49.0	47.5	47.3	44.4**
% survival, post natal day 0		97.7	97.4	99.3	96.6
% survival, post natal day 0 to 1		99.7	98.3	99.0	99.8
% survival, post natal day 1 to 4 (pre-selection)		100.0	98.5	98.7	99.8

% survival, birth to post natal day 4	97.4	94.3	97.1	96.2
% survival, post natal day 4 to 21	96.0	99.0	99.6	100.0
Organ weights (g) for selected weanlings:				
♂:	1.4526	1.4437	1.4370	1.4188
brain	0.2234	0.2088	0.2021	0.1747**
spleen	0.2327	0.2253	0.2053	0.1959**
thymus				
♀:	1.3885	1.3802	1.3788	1.3604
brain	0.2155	0.2050	0.1963	0.1614**
spleen	0.2263	0.2135	0.2093	0.2019
thymus				

* *Statistically significant difference from controls. p<0.05, using Dunnett's test.*

* *Statistically significant difference from controls. p<0.01, using Dunnett's test.*

Based on the results the following NOAELs are proposed:

NOAEL reproductive = 1000 mg/kg bw/day
NOAEL parenteral = 300 mg /kg bw/day based on the pathological finding in livers of females at 600 mg/kg bw/day
NOAEL offspring = 600 mg/kg bw/day based on reduction in mean pup body weight, body weight gain and spleen weight in the 1000 mg/kg bw/day dose group (F1, F2) and the reduction thymus weight (F2)

The NOAELs are the same as were derived during the previous evaluation.

b) <u>Evaluation of additional data for the purpose of renewal of Annex I inclusion</u>

No new studies were submitted for the purpose of renewal of Annex I inclusion.

B.6.6.2. Developmental toxicity studies

<u>a) Previous evaluation (2005-2011)</u>
Data on developmental toxicity in the rabbit were submitted during the EU review of GA4/7 and are available in the EU DAR. The study was considered acceptable in the EFSA conclusion and is considered adequate for supporting renewal of GA4/7; no new developmental toxicity studies are submitted. EFSA (2012) concluded that GA4/7 was not teratogenic in the rabbit, the developmental NOAEL was 300 mg/kg bw/d; this value was used to derive the AOEL. Regulation (EU) No 283/2013 specifies that developmental toxicity should be investigated in both the rabbit and the rat; however no developmental toxicity studies in the rat are available for gibberellins GA4/7. No concerns were raised in the two-generation study in the rat (B6..6.1/01); the study period covered exposure of dams during gestation and subsequent evaluation of offspring therefore signs of marked developmental toxicity would have been noted during this study. It is therefore considered that sufficient data on the potential for GA4/7 to cause developmental toxicity in both rats and rabbits is available, and further developmental toxicity studies are not considered necessary and have not been performed.

B.6.6.2.1. Developmental toxicity study in the rat

Taken from DAR (2005-2011):
No study to investigate teratogenicity in the rat was submitted.

The multigeneration reproduction study in the rat, described above, resulted in a NOAEL for maternal toxicity of 300 mg/kg bw/day and a NOAEL for embryofoetal toxicity of 600 mg/kg bw/day. There were no increases in incidence of any macroscopic malformation at birth in either generation of the multigeneration study, and litter size/offspring survival in the immediate post natal period was similar to controls, indicating that there were no subtle adverse structural effects on foetal development in the rat at dietary levels equivalent to 1000 mg/kg bw/day. A rat teratogenicity study would therefore not be likely to provide any indication of a significant developmental hazard at dose levels up to 1000 mg/kg bw/day.

RMS comments and conclusion:
No rat developmental toxicity studies were submitted during the first evaluation of GA4/7 (2005-2011), and were not considered necessary. The RMS agrees with the applicant, and with the decision taken during the previous evaluation of GA4/7. Thus, no additional studies on developmental toxicity need to be performed.

B.6.6.2.2. Developmental toxicity study in the rabbit

a) Previous evaluation (2005-2011)

PREVIOUS EVALUATION	This study was evaluated in the DAR and has been considered by EFSA.
Data point addressed:	CA 5.6.2/01 (B.6.6.3 DAR)
Author(s) (year):	[redacted] (1989)
Title:	Teratology study with gibberellins A4A7 (GA4A7) in rabbits
Laboratory report / project number:	[redacted]
Testing facility:	[redacted]
Published:	No
Test guideline used:	In-house method which was considered to meet the requirements of OECD 414 and EEC Method B.31
Deviations:	The study was conducted prior to adoption of the guideline therefore there are some minor deviations, for example: test substance administration was over days 7-19 of gestation rather than 6-18 as recommended in OECD 414, and survival was impaired at the highest dose. However an adequate range of dose levels was tested along with a negative control and examination of foetuses was equivalent to the recommendations in the guideline. The study is therefore considered to be acceptable.
GLP:	Yes
EU Agreed Endpoint:	NOAEL 300 mg/kg bw/d

Executive summary
Groups of 18 female rabbits were administered gibberellins by oral gavage at dose levels of 0, 100, 300 or 1000 mg/kg bw/d on days 7 to 19 of gestation. Daily records of bodyweight, food consumption, clinical or physical effects of treatment were recorded. The does were subject to caesarean section on Day 29 and then given a gross internal examination. Viable foetuses were examined for gross abnormalities, weighed, terminated, examined

internally and then processed for skeletal examination. Clinical signs of toxicity (prostration, hypoactivity, unsteady gait, stained urogenital region, voiding no or few faeces and abortion) were observed occasionally in the 1000 mg/kg bw/d group, predominantly during the last week of dosing. The percentage of animals surviving to study termination was 100% for the controls, 94% in the 100 and 300 mg/kg bw/d groups and 22% in the 1000 mg/kg bw/d group. Body weight gain and food consumption were also reduced in the 1000 mg/kg bw/d group. The number of resorptions (early and late) was increased in the 1000 mg/kw bw/d group, and as a consequence the number of live foetuses was reduced. There were no treatment-related foetal external, soft tissue or skeletal abnormalities; there was no evidence of teratogenicity. The NOAEL for maternal toxicity was 300 mg/kg bw/d based on clinical and necropsy observations, and significant reductions in body weight, body weight gain and food consumption at 1000 mg/kg bw/d. The NOAEL for developmental toxicity was 300 mg/kg bw/d based on the increase in the percentage of resorptions and the decrease in the percentage of live foetuses at 1000 mg/kg bw/d.

I Materials and Methods

A. Materials:

1.	**Test Material**	Gibberellins GA4/7
	Description	White powder
	Lot/Batch	16-213-CD
	Purity	90%
	Stability	Assumed to be stable for the duration of the study
2.	**Vehicle**	0.2% hydroxypropylmethylcellulose (HPMC)
3.	**Test animals**	Rabbits
	Species	New Zealand White Hra: (NZW)SPF
	Age	Young adult
	Weight	3.308-3.376 g (females)
	Source	[redacted]
	Acclimation period	21 days
	Diet	Standard Lab rabbit diet (Purina Certified Rabbit Chow #5322) measured daily ration
	Water	Tap water *ad libitum*
	Housing	Animals were individually housed in suspended screen-bottomed stainless steel cages
4.	**Environmental conditions**	
	Temperature	19-24.4°C
	Humidity	30-70%
	Air changes	Not stated
	Photoperiod	Alternating 16 hour light and 8 hour dark cycle

B. Study Design and Methods

1. In life dates: Artificial insemination occurred on 3, 5 10 or 12 January 1989 and was defined as Day 0 of gestation. The study was terminated on gestation Day 29.

2. Animal assignment and treatment

73 rabbits were artificially inseminated, using standard methods, for allocation to four study groups of 18

animals/group. Control rabbits were dosed with the vehicle alone, 0.2% HPMC. The other three groups were dosed with the test substance at 100, 300 or 1000 mg/kg bw/d.

3. Dose preparation

Doses were prepared freshly each week and aliquots frozen until required and thawed on daily basis. Samples were collected regularly throughout the dosing phase for analysis of homogeneity and stability. Dose administration was via oral gavage at a dose volume of 10 mL/kg bw; dose were adjusted for most recently recorded body weight values.

4. In-life observations

Rabbits were observed twice daily for mortality/moribund status and once a day for signs of toxicity. Bodyweights were recorded on Days 0, 7, 10, 13, 16, 20, 24 and 29 of gestation with physical examinations of each rabbit completed at each bodyweight interval. Daily food consumption was also recorded.

5. Post mortem observations

The does were subject to macroscopic necropsy following caesarean section. The gravid uterine weight was recorded after removal and ovaries examined for gross abnormalities. The number of corpora lutea was recorded in each case. After completion of the internal examination of the dam, the uterus was opened, conceptuses removed and placental membranes incised. The number of live and dead foetuses; early and late resorption and any other abnormalities were recorded. The viable foetuses were examined for external abnormalities, weighed and sacrificed and then subjected to soft tissue examination of the thoracic and abdominal cavities, at which time foetal sex was also confirmed. The brain was examined after a mid-coronal slice was made. Viscera were removed and the foetus processed for skeletal examination.

6. Statistics ANOVA or ANCOVA tests were used

II Results and Discussion

A. Clinical signs and mortality

The percent survival for dams was 100%, 94%, 94% and 22% for groups dosed at 0, 100, 300 or 1000 mg/kg bw/d. The one rabbit that died after dosing at 100 mg/kg bw/d, died on Day 14. The one decedent in the 300 mg/kg bw/d group was sacrificed on Day 18 after aborting. In the high dose group, 4 rabbits died between Day 20 and 23 (after cessation of dose administration); 6 rabbits were sacrificed in a moribund condition between Day 17 and 22; four were sacrificed after aborting on Day 17, 18 or 20 (1, 1, 2 animals respectively). Clinical signs of reaction to treatment were limited to the high dose group where prostration, hypoactivity, unsteady gait, stained urogenital region, voiding no or few faeces and abortion were noted on occasion during the study, predominantly in the last week of dose administration.

B. Body weight and food consumption

Body weight, weight gain and food consumption were unaffected by treatment at 100 or 300 mg/kg bw/d, in comparison with controls. In the high dose group, 1000 mg/kg bw/d, bodyweights were significantly lower than controls on Day 13, 16 and 20. Weight gains for all periods between day 7 and day 20 and the overall gain from Day 7-20 were lower than controls. Food consumption was significantly lower for daily intervals from Day 7 to 20 for the high dose group.

C. Reproductive parameters

Pregnancy rates for controls and rabbits dosed at 100, 300 or 1000 mg/kg bw/d were stated in the report to be 94%, 100%, 75% and 100% respectively, although necropsy findings for two females at 1000 mg/kg bw/d included the observation 'Uterus apparently non-gravid'. However, the findings are not considered related to treatment.

In the high dose group, 1000 mg/kg bw/d, there was an increase in percent resorptions (early, late and total) and a consequent decrease in the percent live foetuses. The increased resorption values however, did not achieve statistical significance at the high dose level. Other maternal indicators showed no differences of note between control and treated groups (pre- or post-implantation losses; percent live foetuses; resorptions in low and intermediate groups;

sex ratio {percent live males} or foetal bodyweight).

D. Post mortem findings

Dams

There were no significant differences for gravid uterus weights. The incidence of lung congestion, white foci on the gallbladder, liver adhesions and pale kidneys was elevated in the high dose group. There were few macroscopic changes observed among rabbits dosed at 100 or 300 mg/kg bw/d.

Foetal parameters

There were no treatment–related foetal external abnormalities identified. In the low dose group, one foetus from a single litter had oedema and multiple malformations that included cleft palate, ablepharia and exencephaly. There were no treatment–related foetal visceral abnormalities identified. Isolated incidences of gallbladder agenesis for one control foetus; external hydrocephaly and vessel variations noted in the 300 mg/kg bw/d group and absence of the azygous pulmonary lobe for foetuses in the 0, 100 and 300 mg/kg bw/d groups were not related to treatment. There were no treatment–related foetal skeletal abnormalities identified. Skeletal changes (variations and malformations) occurred among control and treated groups but showed no dose-relationship.

Conclusion

Based on the above; the NOAEL for maternal toxicity was considered to be 300 mg/kg bw/d; the NOAEL for developmental toxicity was considered to be 300 mg/kg bw/d. The endpoints were agreed in the EFSA Conclusions and used for derivation of the AOEL, they are therefore are still considered valid.

RMS comments and conclusion:

The study follows GLP and is comparable to the OECD 414 (1981) as the dosing was performed in the period of organogenesis (day 7 to Day 19). According to the newer version, the tested chemical should be administered at least from implantation to one day prior to the day of scheduled humane killing. The difference between the previous versions of OECD 414 and the one published in 2018 is also the addition of a number of endocrine-related measurements in the dams and in the foetuses, which are rat-specific, thus the new requirements are in the case of rabbit study not relevant. In this study only 18 females/group instead of 20 dams/group with implantation sites at necropsy (required by the current OECD 414) were used. In the study, the mid dose amounted to 14 pregnant females at the scheduled sacrifice, and the high dose group to only 4 survived pregnant rats. No historical control data were available. The study is acceptable with imitations.

Table B.6.6.2.2-1 Mortality in the different dose groups:

	Dose, mg/kg bw/day			
	0	**100**	**300**	**1000**
No. dams	18	18	18	18
No. deaths	0	1	1	14
Reason of death				
found dead	0	1 (day 14)	0	4 (day 20-23)
sacrificed in moribound condition	0	0	0	6 (day 17-22)
sacrificed after aborting	0	0	1 (day 18)	4 (day 17-20))

Treatment related clinical signs were observed only in dams dosed at 1000 mg/kg bw/day which died during the study. The four survived rats did not show any clinical sign. Body weight, body weight gain and food consumption were affected only at 1000 mg/kg bw/day when compared to controls. In this group 78.8% mortality occurred, thus reproductive parameters and foetal findings cannot be used for comparison to other

groups when searching for a dose response relationship or reproducible effects. Thus, in our opinion the highest dose can be used for setting the NOAEL/LOAEL only with limitation e.g. effects seen till the death of females on day 17. The mid dose, 300 mg/kg bw/day with only 13 pregnant females is also questionable; however the number of live foetuses doesn't significantly differ from those found in other two groups, thus this group can be considered in the assessment for setting NOAEL/LOAEL value. Reproductive parameters 0-300 mg/kg bw/day did not seem significantly affected by the treatment; however the values were recalculated recalculated from individual female reproduction data as in the control group the reported value for "Implantations" was lower than for "Live foetuses".

Table B.6.6.2-2 Summary table of maternal and litter parameters and findings

Parameter/Gestational day		**Dose (mg/kg bw/d)**			
		0	**100**	**300**	**1000**
Maternal parameters					
Clinical signs of toxicity		-	-	-	prostration, hypoactivity, unsteady gait, stained urogenital region, no or few faeces, abortion
Maternal bodyweight (kg)	**0**	3.308±0.22	3.341±0.22	3.314±0.18	3.376±0.23
	7	3.556±0.29	3.621±0.28	3.556±0.20	3.631±0.25
	10	3.562±0.31	3.623±0.29	3.558±0.19	3.455±0.24
	13	3.591±0.31	3.663±0.27	3.583±0.20	3.288±0.26**
	16	3.729±0.28	3.742±0.25	3.636±0.20	3.163±0.29**
	20	3.745±0.30	3.788±0.27	3.695±0.22	3.113±0.32**
	24	3.794±0.31	3.865±0.25	3.733±0.21	3.565±0.22
	29	3.768±0.31	3.883±0.26	3.742±0.24	3.675±0.22
Maternal Body weight gain [kg]	**0-7**	0.25±0.13	0.28±0.12	0.24±0.08	0.26±0.10
	7-10	0.01±0.06	0.00±0.05	0.00±0.04	-0.18±0.10**
	10-13	0.03±0.07	0.04±0.06	0.03±0.04	-0.17±0.12**
	13-16	0.14±0.19	0.06±0.09	0.05±0.06	-0.13±0.126**
	15-20	0.02±0.19	0.05±0.05	0.06±0.07	-0.15±0.16**
	20-24	0.05±0.08	0.08±0.09	0.04±0.04	0.13±0.08
	24-29	-0.03±0.16	0.02±0.12	0.01±0.13	0.11±0.02
	7-20	0.19±0.08	0.15±0.11	0.14±0.10	-0.52±0.40**
	0-29	0.46±0.24	0.53±0.19	0.43±0.16	0.42±0.15
Food Consumption (g/animal)	**7-8**	176.1±39.7	167.4±38.7	164.5±16.5	100.1±31.2**
	8-9	202.0±41.0	196.6±39.4	170.9±28.0	60.3±42.7**
	9-10	155.7±59.9	164.9±47.3	172.7±20.6	39.2±30.8**
	10-11	168.0±41.8	164.8±39.8	169.8±27.5	38.2±41.3**
	11-12	168.3±47.8	173.9±31.5	150.3±35.4	35.6±40.6**
	12-13	153.9±49.3	163.0±33.8	135.1±50.1	25.5±39.4**
	13-14	153.3±60.8	158.8±25.6	132.3±45.3	22.5±35.7**
	14-15	158.9±74.9	170.2±29.6	139.6±37.2	23.1±47.7**
	15-16	165.7±57.0	168.0±46.7	149.9±53.1	29.3±48.2**
	16-17	183.8±29.5	168.7±51.9	148.5±51.1	39.6±57.2**
	17-18	184.2±37.7	172.1±52.2	161.0±52.4	43.4±59.3**
	18-19	189.6±22.6	171.4±45.2	171.0±32.9	30.7±50.4**
	19-20	184.7±28.3	167.5±41.1	160.4±37.4	31.2±48.5**
	20-21	174.7±29.6	162.6±41.2	150.2±37.7	104.0±97.8
	21-22	176.6±38.8	154.23±42.1	158.1±37.7	150.8±44.2
	22-23	161.1±58.2	155.51±50.2	158.3±36.1	157.1±37.6
	23-24	134.3±56.3	134.0±37.6	128.1±41.7	168.8±38.4
	24-25	117.2±69.0	130.8±41.6	92.8±47.7	173.9±28.7

	25-26	105.4±72.0	106.6±60.2	88.6±46.5	155.6±29.2
	26-27	82.1±72.1	85.8±53.9	74.48±42.3	155.4±21.1
	27-28	98.3±69.8	112.2±66.0	92.5±46.7	158.6±25.1
	28-29	99.4±64.8	118.83±61.94	91.7±38.2	144.3±24.9
Pregnant (#)		17	18	14	18
Deaths (#)		-	1	1	14
Aborted		0	0	1	6
Dams with viable f (#)		16	16	13	4
Dams with no viable f (#)		1	1	0	0
Litter Parameters (recalculated without stat. analyses from individual female reproduction data)					
Corpora lutea (#)		13.88	15.06	13.77	15.00
Implantations (#)		7.00	7.38	7.85	7.50
Preimplantation loss (%)		49.55	51.04	42.37	50.00
Postimplantation loss (%)		4.46	7.62	4.82	30.00
Resorptions (total)					
- Early embr. death (#)		0.06	0.56	0.23	1.75
- Late embr. death (#)		0.13	0.00	0.23	0.50
Live foetuses (#)		6.69	6.81	7.38	5.25
Percent males (sex ratio)		59.81	51.38	52.08	42.86
Foetal bodyweight (g)		43.72	42.86	44.28	43.88
Gravid uterus weight (g)		394.4	416.4	441.6	355.4

**p<0.05, **p<0.01, # -per litter*

The malformations reported at 100 mg/kg bw/day were considered to be incidental and not treatment related as they were found in the same foetus. In one foetus at 300 mg/kg bw/day hydrocephalus and carotid artery arising from the innominate were observed. Hydrocephalus is a rare malformation and, in this study, it was seen only in one foetus, no other visceral malformations were found in any of the treated groups. The event seems sporadic, unrelated to treatment, however, without having a reliable tested group at the highest dose, the applicant was additionally requested to provide HCD for the two observations found at 300 mg/kg bw/day (the data were not provided in time). The HCD for New Zealand White (NZW) rabbits found in a publication (*Congenital Anomalies* 2012; **52**, 155–161) for studies performed during 2001–2010 report a mean value of 0.33 % with a range 0–1.3% in one laboratory and in another one a men value of 0.05% with a range 0–0.62%.
The effects observed in foetuses lack of statistical significance and dose response when compared between groups 0-300 mg/kg bw/day.

Table B.6.6.2-3 Foetal findings considered different from control value

	Dose Level (mg/kg/day)							
	0		**100**		**300**		**1000**	
External observation								
Litters evaluated	16		15[a]		13		4	
Foetuses evaluated	107		101		96		21	
	NF (%)	NL (%)	NF (%)	NL (%)	NF (%)	NL (%)	NF (%)	NL (%)
Oedema	0 (0.0)	0 (0.0)	1 (1.0)[b]	1 (6.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Cleft palate [M]	0 (0.0)	0 (0.0)	1 (1.0)[b]	1 (6.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Ablepharia [M]	0 (0.0)	0 (0.0)	1 (1.0)[b]	1 (6.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Exencephaly [M]	0 (0.0)	0 (0.0)	1 (1.0)[b]	1 (6.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Total Number affected	**0 (0.0)**	**0 (0.0)**	**1 (1.0)**	**1 (6.7)**	**0 (0.0)**	**0 (0.0)**	**0 (0.0)**	**0 (0.0)**
Visceral tissue observations								
Litters evaluated	16		16		13		4	
Foetuses evaluated	107		109		96		21	

	NF (%)	NL (%)	NF (%)	NL (%)	NF %)	NL (%)	NF (%)	NL (%)
Brain external hydrocephaly M	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.0)[c]	1 (7.7)	0 (0.0)	0 (0.0)
	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.0)[c]	1 (7.7)	0 (0.0)	0 (0.0)
Total Number affected	**5 (4.7)**	**3 (19)**	**3 (2.8)**	**1 (6.3)**	**3 (3.1)**	**2 (15)**	**0 (0.0)**	**0 (0.0)**
Skeletal observations								
Litters evaluated	16		16		13		4	
Foetuses evaluated	107		109		96		21	
	NF (%)	NL (%)	NF (%)	NL (%)	NF (%)	NL (%)	NF (%)	NL (%)
Hyoid arch(es) bent	2 (1.9)	2 (13)	1 (0.9)	1 (6.3)	5 (5.2)	3 (23)	0 (0.0)	0 (0.0)
Skull bones reduced-frontal	0 (0.0)	0 (0.0)	1 (0.9)[b]	1 (6.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Skull bones reduced-parietal	0 (0.0)	0 (0.0)	1 (0.9)[b]	1 (6.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Sternebrae no.5 unossified	2 (1.9)	2 (13)	5 (4.6)	4 (25)	1 (1.0)	1 (7.7)	1 (4.8)	1 (25)
Sternebrae no.6 unossified	5 (4.7)	3 (19)	12 (11)	7 (44)	10 (10)	4 (31)	1 (4.8)	1 (25)
Extra sternebrae	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.0)	1 (7.7)	0 (0.0)	0 (0.0)
7th cervical rib	0 (0.0)	0 (0.0)	2 (1.8)	1 (6.3)	2 (2.1)	2 (15)	0 (0.0)	0 (0.0)
Ribs malformed/misshapen	0 (0.0)	0 (0.0)	1 (0.9)	1 (6.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Pubis unossified	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.0)	1 (7.7)	0 (0.0)	0 (0.0)
Total Number affected	**68 (64)**	**16 (100)**	**81 (74)**	**16 (100)**	**61 (61)**	**13 (100)**	**19 (90)**	**4(100)**

a – one litter was inadvertently examined, b,c- finding in the same foetus, M – malformation, NOTE: A foetus may appear in more than one category, NF = number of foetuses in category (foetal incidence), NL = number of litters in category (foetal incidence).

Based on the study results, the proposed maternal NOAEL for rabbits is 300 mg/kg bw/d, based on maternal mortality, clinical signs, abortions, reduced body weight gain and food consumption at 1000 mg/kg bw/day.

Based on the study results, the proposed development NOAEL for rabbits is ≥ 300 mg/kg bw/d as no treatment related effects have been observed at 300 mg/kg bw/day. Due to high mortality rate of dams, findings at 1000 mg/kg were not considered reliable.

b) <u>Evaluation of additional data for the purpose of renewal of Annex I inclusion</u>

No new studies were submitted for the purpose of renewal of Annex I inclusion.

B.6.7. NEUROTIXICTY

No data are available and none are required. Regulation 283/2012 specifies that neurotoxicity studies shall be performed for active substances with structures that are similar or related to those capable of inducing neurotoxicity or delayed polyneuropathy, and for active substances which induce specific indications of potential neurotoxicity, neurological signs or neuropathological lesions in toxicity studies at dose levels not associated with marked general toxicity. Gibberellins GA4/7 does not possess structural alerts for neurotoxicity; it is not an organophosphate or carbamate, and no potential for neurotoxicity was observed in the available toxicology studies *in vivo*. In addition, gibberellins GA4/7 is a normal component of the human diet due to its presence in fruit and vegetables, therefore studies to investigate neurotoxicity or delayed polyneuropathy are not considered necessary and are not proposed. EFSA (2012) concluded that no further data are required.

B.6.7.1. Neurotoxicity studies in rodents

a) Previous evaluation (2005-2011)

No studies were submitted

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

No new studies were submitted for the purpose of renewal of Annex I inclusion.

RMS comments and conclusion:

The applicant's explanation (see B6.7) is acceptable. No new studies are deemed to be necessary.

B.6.7.2. Delayed polyneuropathy studies

a) Previous evaluation (2005-2011)

No studies were submitted

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

No new studies were submitted for the purpose of renewal of Annex I inclusion.

RMS comments and conclusion:

The applicant's explanation (see B6.7) is acceptable. No new studies are deemed to be necessary.

B.6.8. OTHER TOXICOLOGICAL STUDIES

B.6.8.1. Toxicity studies on metabolites and relevant impurities

a) Previous evaluation (2005-2011)

No studies were submitted

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

No toxicologically relevant metabolites have been identified therefore, studies of metabolites are not required.

RMS comments and conclusion:

The applicant's explanation is acceptable. No new studies are deemed to be necessary.

B.6.8.2. Supplementary studies on the active substance

a) Previous evaluation (2005-2011)

No studies were submitted

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

Supplementary studies are required by Regulation (EU) No 283/2013 where they are necessary to further clarify effects seen in the standard toxicological studies. As no concerns have been raised in the available standard

studies, supplementary studies are not required and have not been performed. No additional information was identified in the recent literature search.

RMS comments and conclusion:

The applicant's explanation is acceptable. No new studies are deemed to be necessary.

B.6.8.3. Studies on endocrine disruption

RMS comments:

B.6.8.3.1. *Gathering information*

In June 2018, EFSA and the European Chemicals Agency (ECHA) published a guidance for the identification of endocrine disruptors in biocide and pesticide regulations. As the endocrine disruption (ED) criteria and subsequent the ECHA/EFSA guidance document (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), need be applied for all substances for which a decision on approval or renewal of approval is pending on 10/11/2018 (see Minutes of the 23rd meeting Network on Pesticide Steering,) the applicant was requested to provide an updated assessment in line with the guidance document. The ED assessment was received in the middle of February 2019 using the first version of the excel template (Appendix E1). An updated version using the latest version of Appendix E1 ffrom February 2019 was received in the middle of April 2019.

Utilising the available toxicological 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 toxicological studies according to the abovementioned guidance utilising Appendix E1 and the corresponding sub-guidance document for completion of the Excel spreadsheet. Appendix E1 is provided as an Appendix to Vol 1.

After a review of scientific peer-reviewed open literature no papers were found that were considered to be relevant. A single concept search strategy was utilized. The RMS found several shortcomings in the summary of the literature search. From the applicants report it is not clear if the literature search was performed covering the period from 1 April 2016 to 31 October 2018 or a period from 1 April 2016 to 31 November 2017. Irrespective of which of the two periods was searched, both search periods are insufficient as stated in the abovementioned guidance literature search has to be performed for the last 10 years. In the literature search studies providing information that supports the existing regulatory data package were considered as non-relevant with the explanation that there is no need to include papers in the Literature Report which do not add new information. However, the guidance document for identification of endocrine disruptors states that all available evidence needs to be taken into consideration. One of the used search terms was benalaxyl. The applicant explained, that benalaxyl was a search term recommended for inclusion by their scientific specialists as being used as a reference substance in the EFSA/ECHA guidance document (FSA Journal 2018; 16(6): 5311). The search parameters for substance names and synonyms were limited only to the CAS numbers of GA3, GA4, GA7 and GA4/7. The searched terms for endocrine disruption were also limited to few words. The RMS concludes that endocrine disrupting properties of gibberellins (GA4/7) were not sufficiently investigated in the provided literature search. The applicant is advised to look into the example reported in Appendix F of the EFSA ED guidance.

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.

Applicants report:

(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 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 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.

B.6.8.3.2. *ED assessment for T-modality*

RMS comments and conclusion:

B.6.8.3.2.1. Have T-mediated parameters been sufficiently investigated?

	Sufficiently investigated
T-mediated parameters	**No,** based on the lack of a long term or carcinogenicity study and the lack of investigations performed in following studies in which thyroid adversity is addressed: - OECD TG 408 (1981) - ID: 1* - OECD TG 409 (1998) - ID: 2 - Comparable to OECD TG 416 (1983, 2001) - ID: 3** - Comparable to OECD TG 414 (1981) - ID: 4***

* no analyses of serum/plasma hormones (T4, T3, TSH, FSH, LH, oestradiol, testosterone) and HDL, LDL, thyroid not weighted

**thyroid of parenteral animals was not weighted

*** the study was done on rabbits, thus the new requirements from 2018 on endocrine-related measurements are no relevant

There is no carcinogenicity or long-term study available, thyroid and relevant hormones were not investigated in the 2-generational study and developmental studies (only 1 study in rabbits provided). Based on the missing information above and the limitations of presented studies the overall database is not considered adequate for the assessment of thyroid adversity.

B.6.8.3.2.2. Lines of evidence for adverse effects and endocrine activity related to 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
2	Sensitive to, but not diagnostic of, EATS	Adrenals histopathology	Dog	13	Weeks	Oral	1100	mg/kg bw/day	Change	1/2 dogs was treated with steroids.	not reliable result	Isolated effects upon adrenals in one dog study, brain weight in one rat study and litter parameters in one rabbit study were related to generalised systemic toxicity at the high dose level only and were unrelated to ED mediated effects.	Overall. No evidence for T-adversity - Datased not sufficiently invenstigated
1		Brain weight	Rat	13	Weeks	Oral	25000	ppm	Increase	Effect seen at discontinuation of treatment and persisted following 4-week recovery period;	Not necessarily indicative of a treatment - related effect and instead may be indicative of adaptation to general toxicity and preservation of key organ functions		

4		Number of live births	Rabbit	13	Days	Oral	1000	mg/kg bw/day	Decrease	not reliable result	Due to high mortality rate of dams at the top dose in the rabbit study the two reproductive effects are not reliable	Due to high mortality rate of dams in the rabbit study the two reproductive effects are not reliable	
4		Post implantation loss	Rabbit	13	Days	Oral	1000	mg/kg bw/day	Increase	not reliable result			
1	Target organ toxicity	Kidney histopathology	Rat	13	Weeks	Oral	25000	ppm	Change	chronic inflammation that tended to be more severe and more prevalent in males; severity in both sexes tended to be reduced following 4-week recovery period.	Treatment related findings in organ weight and histopathology are indicative of potentially adaptive changes and generalised systemic toxicity unrelated to endocrine-mediated activity. Effects upon primary lymphoid tissues (eg thymus and spleen) could be indicative of stress secondary to general systemic	Sufficient evidence of systemic toxicity kidney (dog, rat), liver (rat) and spleen (rat), possible effect on thymus toxicity (dog)	
1			Rat	13	Weeks	Oral	25000	ppm	Change	Rough surface (more prevalent in females) or depressed foci/areas in the cortex (more prevalent in males); changes were largely unresolved following 4-week			

										recovery period.	toxicity.		
3			Rat	5	Months	Oral	600	mg/kg bw/day	Increase	DR increases in the incidence and severity of nephropathy, medullary tubular dilatation, medullary fibroplasia, medullary basophilic interstitium and medullary tubule hyperplasia. Medullary papillary urothelial hyperplasia also apparently observed.			
3			Rat	5	Months	Oral	600	mg/kg bw/day	Increase				
3			Rat	5	Months	Oral	600	mg/kg bw/day	Increase				
1		Kidney weight	Rat	13	Weeks	Oral	25000	ppm	Decrease	stst. Sign. relative in males, not stat. Sig. Absolute in females			
1			Rat	13	Weeks	Oral	25000	ppm	Increase				
2			Dog	13	Weeks	Oral	1100	mg/kg bw/day	Increase	stst. Sign. relative			
3			Rat	5	Months	Oral	1000	mg/kg bw/day	Decrease	stst. Sign. Abolute (males)			

1		Liver histopathology	Rat	13	Weeks	Oral	25000	ppm	Change	epatocellular vacuolization (high incidence, slight to moderate severity) and hepatocellular degeneration (some evidence) were largely resolved following 4-week recovery period			
1		Liver weight	Rat	13	Weeks	Oral	25000	ppm	Decrease	not consistnet effect between males and females			
1			Rat	13	Weeks	Oral	25000	ppm	Increase				
2			Dog	13	Weeks	Oral	1100	mg/kg bw/day	Increase	stst. Sign. Relative, absolute			
3		Spleen weight	Rat	Unknown. Animals exposed from conception.	Months	Oral	1000	mg/kg bw/day	Decrease	observed in F1 and F2 pups			
2		Thymus histopathology	Dog	13	Weeks	Oral	1100	mg/kg bw/day	Change	atrophy, 3/4 males, reported to be a result of stress			
2		Thymus weight	Dog	13	Weeks	Oral	1100	mg/kg bw/day	Decrease	obsreved as small (males)			

3			Rat	Unknown. Animals exposed from conception.	Months	Oral	1000	mg/kg bw/day	Decrease	stat. Signif absolute, F2 offspring males			
1	Systemic toxicity	Clincial chemistry	Rat	13	Weeks	Oral	25000	ppm	Change	Effect noted alongside several other signs of generalised toxicity not indicative of an ED effect	Treatment related findings such as reduced bodyweight, occasional alterations in food intake, haematological or clinical chemistry parameters, clinical signs etc. are considered a consequence of general systemic toxicity and were unrelated to endocrine mediated activity.		
1			Rat	13	Weeks	Oral	25000	ppm	Change				
1		Body weight	Rat	13	Weeks	Oral	50000	ppm	Decrease				
2			Dog	13	Weeks	Oral	1100	mg/kg bw/day	Decrease				
3			Rat	5	Months	Oral	1000	mg/kg bw/day	Decrease				
3			Rat	5	Months	Oral	600	mg/kg bw/day	Decrease				
3			Rat	5	Months	Oral	1000	mg/kg bw/day	Decrease				
3			Rat	Unknown. Animals exposed from conception.	Months	Oral	1000	mg/kg bw/day	Decrease				
4			Rabbit	13	Days	Oral	1000	mg/kg bw/day	Decrease				
1		Clinical chemistry and haematology	Rat	13	Weeks	Oral	25000	ppm	Change				
1		Clinical signs	Rat	13	Weeks	Oral	25000	ppm	Change				
1			Rat	13	Weeks	Oral	50000	ppm	Change				
2			Dog	13	Weeks	Oral	1100	mg/kg bw/day	Change				
4			Rabbit	13	Days	Oral	300	mg/kg bw/day	Increase				
1		Food consumption	Rat	13	Weeks	Oral	50000	ppm	Decrease				
2			Dog	13	Weeks	Oral	1100	mg/kg bw/day	Decrease				
3			Rat	5	Months	Oral	1000	mg/kg bw/day	Increase				
1		Mortality	Rat	13	Weeks	Oral	25000	ppm	Decrease				

4			Rabbit	13	Days	Oral	100	mg/kg bw/day	Increase				

B.6.8.3.2.3. Assessment of the integrated lines of evidence and weight of evidence for T-mediated adversity and endocrine activity

WoE for T-mediated adversity:

-Thyroid weight and histopathology were measured only in a short-term oral toxicity studies in dog (ID: 2) and no adverse changes were observed.

- In ID: 1, 3, no macroscopic changes on thyroid were observed

WoE for T-mediated endocrine activity:

No data available.

B.6.8.3.2.4. Initial analysis of the evidence and identification of relevant scenario for the ED assessment of T-modality

Adversity based on T-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 "**T-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 **T-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	

B.6.8.3.2.5. Conclusion of the assessment of T-modality

The overall WoE suggests that T-mediated parameters have not been sufficiently investigated and T-mediated adversity was not observed. Therefore, missing information according to scenario 2a (iii) should be generated.

As there are no specific studies which can be additionally submitted to cover the endocrine activity of the thyroid gland. The RMS is of the opinion that according to the data provided, no final conclusions can be reached regarding the thyroid mediated adversity and endocrine activity. In case of a negative conclusion about EAS

modalities, a combined chronic toxicity and carcinogenicity study (OECD 451-3) and extended one-generation reproductive toxicity study (OECD 443) are proposed.

B.6.8.3.3. *ED assessment for EAS-modalities*

B.6.8.3.3.1. Have EAS-mediated parameters been sufficiently investigated?

	Sufficiently investigated
EAS-mediated parameters	**No,** based on the lack of investigations performed in the following studies: - OECD TG 408 (1981) - ID: 1 - Comparable to OECD TG 416 (1983, 2001) - ID: 3

ID:1 Not weighted: epididymis, prostate + seminal vesicles with coagulating glands, uterus, the histopathology was not performed for: male mammary glands, coagulating glands, and vaginal smear; were not specifically mentioned; no sperm measures (cauda epididymis sperm reserves, sperm motility, sperm morphology).
ID: 3 F1 Compared to OECD 416 (201) the following EAS mediated parameters were not investigated: histopathology of vagina, uterus with cervix, ovaries, one testis, one epididymis, seminal vesicles, prostate and coagulating gland was performed on approximately 10 parenteral rats/sex/group and not on all parenteral animals selected for mating in the control and high dose group. The exception was the histopathological investigation of right testis in the F0 generation which were examined in all the parenteral animals in the control and high dose group (30 animals/dose.). Sperm parameters and organ weights were investigated in all 30 males.

B.6.8.3.3.2. Lines of evidence for adverse effects and endocrine activity related to 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
5	In vitro mechanistic	Androgen receptor	Human	40	Hours	Uptake from the medium	>10000	other	No effect	No effect	NO ER and AR mediated (ant)agonistic activity. Supporting information. The study was assessed as not reliable.	Overall not sufficient to show absence of endocrine activity.	A
5		Estrogen receptor	Human	40	Hours	Uptake from the medium	>10000	other	No effect	No effect			E
1	EATS-mediated	Ovary weight	Rat	13	Weeks	Oral	25000	ppm	Increase	Effect noted at top dose only at which there was clear evidence of generalised toxicity, significant decrease in body weight)	Ovary and testis related weights were affected in one study at a very high dose level, were not replicated and are likely to be secondary to generalised toxicity at this treatment level.	Overall not sufficient to show absence of endocrine activity.	Overall. No evidence for EAS-adversity - Datased not sufficientl y invenstiga ted
1		Testis weight	Rat	13	Weeks	Oral	25000	ppm	Increase				

2		Adrenals histopathology	Dog	13	Weeks	Oral	1100	mg/kg bw/day	Change	1/2 dogs was treated with steroids.	not reliable result	
1	Sensitive to, but not diagnostic of, EATS	Brain weight	Rat	13	Weeks	Oral	25000	ppm	Increase	Effect seen at discontinuation of treatment and persisted following 4-week recovery period;	Not necessarily indicative of a treatment -related effect and instead may be indicative of adaptation to general toxicity and preservation of key organ functions	Isolated effects upon adrenals in one dog study, brain weight in one rat study and litter parameters in one rabbit study were related to generalised systemic toxicity at the high dose level only and were unrelated to ED mediated effects. Due to high mortality rate of dams in the rabbit study the two reproductive effects are not reliable
4		Number of live births	Rabbit	13	Days	Oral	1000	mg/kg bw/day	Decrease	not reliable result	Due to high mortality rate of dams at the top dose in the rabbit study the two reproductive effects are not reliable	
4		Post implantation loss	Rabbit	13	Days	Oral	1000	mg/kg bw/day	Increase	not reliable result		

1	Target organ toxicity	Kidney histopathology	Rat	13	Weeks	Oral	25000	ppm	Change	chronic inflammation that tended to be more severe and more prevalent in males; severity in both sexes tended to be reduced following 4-week recovery period.	Treatment related findings in organ weight and histopathology are indicative of potentially adaptive changes and generalised systemic toxicity unrelated to endocrine-mediated activity. Effects upon primary lymphoid tissues (eg thymus and spleen) could be indicative of stress secondary to general systemic toxicity.	Sufficient evidence of systemic toxicity kidney (dog, rat), liver (rat) and spleen (rat), possible effect on thymus toxicity (dog)	
1			Rat	13	Weeks	Oral	25000	ppm	Change	Rough surface (more prevalent in females) or depressed foci/areas in the cortex (more prevalent in males); changes were largely unresolved following 4-week recovery period.			
3			Rat	5	Months	Oral	600	mg/kg bw/day	Increase	DR increases in the incidence and severity of nephropathy, medullary tubular dilatation, medullary fibroplasia, medullary basophilic interstitium and medullary tubule hyperplasia. Medullary papillary urothelial hyperplasia also apparently observed.			
3			Rat	5	Months	Oral	600	mg/kg bw/day	Increase				
3			Rat	5	Months	Oral	600	mg/kg bw/day	Increase				
1		Kidney weight	Rat	13	Weeks	Oral	25000	ppm	Decrease	stst. Sign. relative in			

1			Rat	13	Weeks	Oral	25000	ppm	Increase	males, not stat. Sig. Absolute in females			
2			Dog	13	Weeks	Oral	1100	mg/kg bw/day	Increase	stst. Sign. relative			
3			Rat	5	Months	Oral	1000	mg/kg bw/day	Decrease	stst. Sign. Abolute (males)			
1		Liver histopathology	Rat	13	Weeks	Oral	25000	ppm	Change	epatocellular vacuolization (high incidence, slight to moderate severity) and hepatocellular degeneration (some evidence) were largely resolved following 4-week recovery period			
1		Liver weight	Rat	13	Weeks	Oral	25000	ppm	Decrease	not consistnet effect between males and females			
1			Rat	13	Weeks	Oral	25000	ppm	Increase				
2			Dog	13	Weeks	Oral	1100	mg/kg bw/day	Increase	stst. Sign. Relative, absolute			
3		Spleen weight	Rat	Unknown. Animals exposed from conception.	Months	Oral	1000	mg/kg bw/day	Decrease	observed in F1 and F2 pups			
2		Thymus histopathology	Dog	13	Weeks	Oral	1100	mg/kg bw/day	Change	atrophy, 3/4 males, reported to be a result of stress			

2		Thymus weight	Dog	13	Weeks	Oral	1100	mg/kg bw/day	Decrease	obsreved as small (males)			
3			Rat	Unknown. Animals exposed from conception.	Months	Oral	1000	mg/kg bw/day	Decrease	stat. Signif absolute, F2 offspring males			
1	Systemic toxicity	Clincial chemistry	Rat	13	Weeks	Oral	25000	ppm	Change	Effect noted alongside several other signs of generalised toxicity not indicative of an ED effect	Treatment related findings such as reduced bodyweight, occasional alterations in food intake, haematological or clinical chemistry parameters, clinical signs etc. are considered a consequence of general systemic toxicity and were unrelated to endocrine mediated activity.		
1			Rat	13	Weeks	Oral	25000	ppm	Change				
1		Body weight	Rat	13	Weeks	Oral	50000	ppm	Decrease				
2			Dog	13	Weeks	Oral	1100	mg/kg bw/day	Decrease				
3			Rat	5	Months	Oral	1000	mg/kg bw/day	Decrease				
3			Rat	5	Months	Oral	600	mg/kg bw/day	Decrease				
3			Rat	5	Months	Oral	1000	mg/kg bw/day	Decrease				
3			Rat	Unknown. Animals exposed from conception.	Months	Oral	1000	mg/kg bw/day	Decrease				
4			Rabbit	13	Days	Oral	1000	mg/kg bw/day	Decrease				
1		Clinical chemistry and haematology	Rat	13	Weeks	Oral	25000	ppm	Change				
1		Clinical signs	Rat	13	Weeks	Oral	25000	ppm	Change				
1			Rat	13	Weeks	Oral	50000	ppm	Change				
2			Dog	13	Weeks	Oral	1100	mg/kg bw/day	Change				
4			Rabbit	13	Days	Oral	300	mg/kg bw/day	Increase				

1		Food consumption	Rat	13	Weeks	Oral	50000	ppm	Decrease				
2			Dog	13	Weeks	Oral	1100	mg/kg bw/day	Decrease				
3			Rat	5	Months	Oral	1000	mg/kg bw/day	Increase				
1		Mortality	Rat	13	Weeks	Oral	25000	ppm	Decrease				
4			Rabbit	13	Days	Oral	100	mg/kg bw/day	Increase				

B.6.8.3.3.3. Assessment of the integrated lines of evidence and weight of evidence for T-mediated adversity and endocrine activity

WoE for EAS-mediated adversity:

-In rats (ID:3), no treatment-related effect was observed in males in testes weight ,testes histopathology, prostate weight and histopathology, epididymis weight and histopathology, seminal vesicles histopathology and coagulating gland histopathology. In females, no treatment-related effects were observed in mammary gland, ovary and uterus histopathology, ovary weight and histopathology, uterus weight and histopathology vagina and cervix histopathology (ID: 14). No effects on pituitary gland histopathology were observed in both males and females. In rats (ID:1), Increased ovary and testis weights were observed in a single study in rats. However, the effects were measured at only a high treatment level associated with generalised systemic toxicity and los of body weight.

- In dogs (ID:2), no treatment-related effect was observed in males in testes (with epididymis) weight, testes, epididymis and prostate histopathology and epididymis weight, and in females in ovary and uterus (with cervix) weight and histopathology and in histopathology of vagina.

WoE for EAS-mediated endocrine activity

- In the *in vitro* study (ID:5) a lack of a positive effect upon the oestrogen or androgen receptors is seen, however, the study was assessed as not reliable and used for supporting information.

B.6.8.3.3.4. Initial analysis of the evidence and identification of relevant scenario for the ED assessment of EAS-modalities

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 "**EAS-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 **EAS-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	Yes/No	2b	Perform MoA analysis	

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)*
investigated)				

B.6.8.3.3.5. Conclusion of the assessment of EAS-modalities

Based on scenario 2a (iii), the endocrine activity was not sufficiently investigated for the EAS-modalities:

- E modality: There is not ToxCast ER model neither Uterotrophic assay (OECD TG 440)
- A modality: There is no Hershberger Assay (OECD TG 441)
- S modality: There is no Steroidogenesis Assay (OECD TG 456)

Therefore, according to the guidance, additional information should be generated (Scenario 2a(iii)). Level 3 studies are required for E modality i.e. OECD TG 440 and A modality i.e. OECD TG 441, and Level 2 study for S modality i.e. OECD TG 456.

- If the above studies are negative, the scenario 2a(ii) applies and ED criteria are not met.
- If endocrine activity is observed, the scenario 2a(i) applies and further data will be needed to support the MoA analysis, i.e. extended one-generation study (OECD TG 443, Level 5).

B.6.8.3.4. *Overall conclusion on the ED assessment for humans*

For the EATS modalities, although adversity was not observed, the dataset was not sufficient for both, adversity and endocrine activity. Therefore, further data need to be generated before a conclusion on whether the ED criteria are met for the EATS-modalities can be drawn.

In the dossier the applicant also pointed out that:

-Gibberellins are ubiquitous in higher plants. The natural occurrence of gibberellins was reviewed by Macmillan, 2002. This publication is summarized 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 significant difference between naturally occurring "residues" of GA4/7 and those derived from the representative agronomic use on pome fruit.

The RMS is aware that according to the ED guidance (EFSA Journal 2018; 16(6):5311) the potential ED hazard of active substances needs to be estimated, however in view of human exposure (through the diet) to residues of GA4/7 being at background levels, maybe a more pragmatic approach not requiring the generation of additional (*in vivo*) data should be considered.

B.6.9. MEDICAL DATA AND INFORMATION

B.6.9.1. Medical surveillance on manufacturing plant personnel and monitoring studies

Manufacturing plant personnel are monitored annually. Medical exams are performed annually or every 3 years dependent upon role, and include spirometry, complete blood count and blood chemistry. No adverse reactions have been documented or reported and there have been no medical surveillance abnormalities to date (approximately 30 years).

B.6.9.2. Data collected on humans

No data are available.

B.6.9.3. Direct observation

No direct observation e.g. clinical cases, adverse reactions or poisoning incidents have been reported.

B.6.9.4. Epidemiological studies

No epidemiological studies on GA4/7 are available.

B.6.9.5. Diagnosis of poisoning (determination of active substance, metabolites), specific signs of poisoning, clinical test

No cases of poisoning have been reported therefore; specific diagnostic tests have not been developed

B.6.9.6. Proposed treatment: first aid measures, antidotes, medical treatment

In the case of exposure with GA4/7 treat symptomatically. Adverse reactions have not been observed in production plant personnel therefore no specific therapeutic regimens have been developed. No cases of poisoning have been reported.

RMS comments and conclusion:

Proposed treatment: first aid measures, antidotes, medical treatment

General measures: Take proper precautions to ensure your own safety before attempting rescue. Remove source of contamination or move victim to fresh air or well-ventilated space. Keep the victim warm. If unconscious place the victim in a head down left sided position. In case of airway obstruction, absent breathing or absent pulse start cardiopulmonary resuscitation following the standard ABC procedure: clear and maintain airway, begin artificial respiration and/or begin cardiac massage.

Inhalation exposures: Remove source of contamination or move victim to the fresh air or well-ventilated space. If transfer is not possible ventilate the room as best as possible and follow general measures.

Skin exposures: Remove contaminated clothing and shoes. Wash well with water and soap. If necessary, obtain medical advice. Wash the clothing prior to the next use.

Eye exposures: Flush with water and allow water to flow gently while holding the eyelids open with a thumb and index finger. If irritation persists immediately obtain medical advice.

Oral exposures: If the victim is awake and able to swallow, rinse the mouth with water and give 200 ml of water. Do not induce vomiting. Never give anything by mouth if the victim is rapidly loosing consciousness or is unconscious. Do not give anything to drink to an unconscious person. Obtain medical advice. If possible, give the doctor product instructions for use.

Note to physicians: Treatment is symptomatic and supportive. Do not perform gastric lavage unless advised so by the Poisons Control centre. Give activated charcoal only if told so by the Poisons Control centre.

B.6.10. REFERENCES RELIED ON

Summary of the public literature search

Two searches were undertaken for relevant literature in the public domain on the active substance gibberellins (GA47 and relevant synonyms). The initial search was undertaken in April and May 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).

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.

RMS comments and conclusion:

The literature search was performed for both GA3 and GA4/7 at once since the applicant expected that there would be overlap of relevant papers. Only searches in bibliographic databases were undertaken. The public literature search process is documented according to 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. The first public literature search was performed on April 2016 and update on May 2016 due to the finding of too few references for Residues and Toxicology/Human Health. An additional search was carried out in November 2017 due to the extension of the submission date for the renewal dossier. The search period is in line with the requirements of the Commission Implementing Regulation (EU) No 844/2012, as referred in Article 8(5) of Regulation (EC) No 1107/2009.

The search strategies were based on a single concept search. The search was performed combining the terms gibberellic acid or GA3 or gibberellin or GA4/7 or using the belonging CAS Registry numbers and applying them to each of the search terms listed by scientific area (Physical Chemistry, Residues, Toxicology, Environmental fate, Ecotoxicology) the "AND" operator. The summary record retrieved were reported for all the scientific area and searched databases together.

The selected Relevance Criteria for the data requirements regarding the Toxicology and metabolism studies werw:

1. Well-defined test material (including its purity and impurity profile).

2. Relevant test species (to the mammalian toxicological assessment -preferred species are rodents - rats and mice, the dog is the preferred non-rodent species).
3. Number of animals per group sufficient to establish a statistical significance.
4. Several dose levels tested (at least 3), preferably including a negative control, to establish a dose-response.
5. Relevant route of administration in terms of risk assessment (oral, dermal or by inhalation).
6. Description of the observations, examinations, analysis performed, or necropsy.
7. Well described test methodology – appropriate guideline referenced
8. In addition: studies which may be helpful for the interpretation of other studies present in the dossier, but do not fit under a specific toxicological endpoint.

In the opinion of the RMS the choice of relevance criteria number 7 is not suitable as it reflects more a consideration of study reliability than relevance. Therefore, in our opinion the non-inclusion of studies in the dossier being "non-GLP" and/or "non-guideline" is not a valuable argument.

Regarding mammalian toxicology, databases Toxcenter (Toxicology Center Database), Medline, CAplus and BIOSIS were searched for the period 1st January 2005 to 6th May 2016 and 1st April 2016 to 14th November 2017. However, Medline, CAPlus and BIOSIS are part of Toxcenter as subfiles. In the second search two additional Toxicology search terms were included (Phototox? and "Mechanistic test for endocrine disruption"). In the RMS opinion the search term for "Mechanistic test for endocrine disruption" is too specific and the search of literature should be repeated using less restrictive terms. The applicant evaluated the possible endocrine disruption properties of GA4/7 according to the latest Guidance for the identification of endocrine disruptors in the context of Regulation (EU) NO 582/2012 and (EC) No 1107/2009. EFSA Journal 2018; 16(6): 5311, 135. However, the additional search performed for ED properties was judged to be not acceptable (see also point B.6.8.3) and should be repeated.

The search from 1st January 2005 to 6th May 2016 does not include any terms for phototoxicity. It is one of the data requirements, however, based on the UV spectra of GA4//, a phototoxicity study is not required. Thus, in our opinion the search can be omitted).

In the Literature review Report on GA4/7, there is stated that the reliability assessment for relevant studies was done according to Klimisch *et al.* (Klimisch, HJ, Andreae E, and Tillmann, U. 1997. A systematic approach for evaluating the quality of experimental toxicological and ecotoxicological data). However, the reliability assessment (in table provided as "Reason(s) for not including this study in the dossier« lacks of Klimisch scores (e.g. 1,2,3,4) with description (e.g. not reliable).

To conclude, according to the search performed by the applicant no data in mammalian toxicology area published in the literature over the last 10 years considered reliable for the risk assessment of gibberellins (GA4/7) and thus not included in the dossier and furthermore in the draft RAR. However, the RMS is of the opinion that the applicant should repeat the literature search and more accurately evaluate the outputs (for endocrine disruptor properties).

Data Point	Author(s)	Year	Title Compagny 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
KCA 5.1.1/01	[redacted]	2000	Absorption, Distribution, Metabolism and Excretion of [14C]-gibberellins GA4GA7 in rats Report No. [redacted] GLP Unpublished	Y	N	-	Valent BioSciences	In DAR (2011) B.6.1.1-1 IIA 5.1.1/01
KCA 5.2.1/01	[redacted]	1997a	GA4/7 99% technical: Acute oral toxicity to the rat Report No. [redacted] GLP Unpublished	Y	N	-	Fine Agrochemicals Ltd	In DAR (2011) B.6.2.1.1 IIA 5.2
KCA 5.2.1/02	[redacted]	1988a	Acute oral toxicity study of Gibberellins A4A7	Y	N	-	Valent BioSciences	In DAR (2011) B.6.2.1.1

			(GA4A7) in rats Report No. [redacted] GLP Unpublished					IIA 5.2.1/01
KCA 5.2.2/01	[redacted]	1997b	GA4/7 99% technical: Acute dermal toxicity to the rat Report No. [redacted] GLP Unpublished	Y	N	-	Fine Agrochemicals Ltd	In DAR (2011) B.6.2.1.2 IIA 5.2
KCA 5.2.2/02	[redacted]	1988b	Acute dermal toxicity study of Gibberellins A4A7 (GA4A7) in rats Report No. [redacted]	Y	N	-	Valent BioSciences	In DAR (2011) B.6.2.1.2 IIA 5.2.2/01

			GLP Unpublished					
KCA 5.2.3/01	[redacted]	1997	Acute inhalation toxicity to rats of GA4/7 99% technical Report No. [redacted] GLP Unpublished	Y	N	-	Fine Agrochemicals Ltd	In DAR (2011) B.6.2.1.3 IIA 5.2
KCA 5.2.3/02	[redacted]	1988	Acute inhalation toxicity study with gibberellins A4A7 (GA4A7) in the rat Report No. [redacted] GLP Unpublished	Y	N	-	Valent BioSciences	In DAR (2011) B.6.2.1.3 IIA 5.2.3/01
KCA 5.2.4/01	[redacted]	1997a	GA4/7 99% technical: Skin irritation to the rabbit Report No. [redacted]	Y	N	-	Fine Agrochemicals Ltd	In DAR (2011) B.6.2.1.4 IIA 5.2

			[redacted] GLP Unpublished					
KCA 5.2.4/02	[redacted]	1988c	Primary dermal irritation study of Gibberellins A4A7 (GA4A7) in rabbits Report No. [redacted] GLP Unpublished	Y	N	-	Valent BioSciences	In DAR (2011) B.6.2.1.4 IIA 5.2.4/01
KCA 5.2.5/01	[redacted]	1997a	GA4/7 99% technical: Eye irritation to the rabbit Report No. [redacted] GLP Unpublished	Y	N	-	Fine Agrochemicals Ltd	In DAR (2011) B.6.2.1.5 IIA 5.2
KCA 5.2.5/02	[redacted]	1988d	Primary eye irritation study of Gibberellins A4A7 (GA4A7) in rabbits Report No. [redacted]	Y	N	-	Valent BioSciences	In DAR (2011) B.6.2.1.5 IIA 5.2.5/01

			[redacted] GLP Unpublished					
KCA 5.2.6/01	[redacted]	1997	GA4/7 99% technical: Skin sensitisation in the guinea pig Report No. [redacted] GLP Unpublished	Y	N	-	Fine Agrochemicals Ltd	In DAR (2011) B.6.2.1.6 IIA 5.2
KCA 5.2.6/02	[redacted]	1988e	Dermal sensitization study of Gibberellins A4A7 (GA4A7) in guinea pigs (Maximization test) Report No. [redacted] GLP Unpublished	Y	N	-	Valent BioSciences	In DAR (2011) B.6.2.1.6 IIA 5.2.6/01

KCA 5.2.6/03	[redacted]	1994	Gibberellin A4/A7: Skin sensitization to the guinea pig Report No. [redacted] GLP Unpublished	Y	N	-	Valent BioSciences	In DAR (2011) B.6.2.1.6 IIA 5.2.6/02
KCA 5.2.7/01	Gerbeix, C.	2018	Gibberellic Acid A4/A7: 3T3 NRU Phototoxicity Test Report No. 45159 TIP CiToxLAB France, BP 563, 27005 Evreux, France GLP Unpublished	N	Y	New study for the purposes of renewal	GA4A7 Task Force	-
KCA 5.3.2/01	[redacted].	1990	13-Week Dietary Toxicity Study with Gibberellins A4/A7 in Rats Report No. [redacted] GLP Unpublished	Y	N	-	Valent BioSciences	In DAR (2011) B.6.3.1.2 IIA 5.3.2/01
KCA 5.3.2/02	[redacted]	2001	ABG-3192 13-Week Oral (Capsule) Toxicity Study	Y	N	-	Valent BioSciences	In DAR (2011) B.6.3.1.2

			in Dogs Report No. [redacted] GLP Unpublished					IIA 5.3.3/01
KCA 5.4.1/01	May, K.	1997	GA4/7: Bacterial mutation assay Report No. 96/FNA024/0935 Huntingdon Life Sciences Ltd., Eye, Suffolk, IP23 7PX, England GLP Unpublished	N	N	-	Fine Agrochemicals Ltd	In DAR (2011) B.6.4.1.1 IIA 5.4
KCA 5.4.1/02	Lawlor, T.E.	1988	Salmonella/mammalian-microsome plate incorporation mutagenicity assay (Ames test) with a confirmatory assay Report No. T8201.501014 Microbiological Associates, Inc., 9900 Blackwell Road, Rockville, MD 20850 GLP Unpublished	N	N	-	Valent BioSciences	In DAR (2011) B.6.4.1.1 IIA 5.4.1/01
KCA 5.4.1/03	Kitching, J.D.	1997	GA4/7 Metaphase chromosome analysis of human	N	N	-	Fine Agrochemicals Ltd	In DAR (2011) B.6.4.1.2 IIA 5.4

			lymphocytes cultured in vitro Report No. FNA 25/962243 Huntingdon Life Sciences Ltd., P.O. Box 2, Huntingdon, Cambridgeshire, PE18 6ES, England GLP Unpublished					
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