European Commission

Draft Renewal Assessment Report prepared according to the Commission Regulation (EU) N° 1107/2009

TRITICONAZOLE

Volume 3 – B.9 (AS)

Rapporteur Member State: Austria

Co-Rapporteur Member State: United Kingdom

Version History

When	What
2003/September	Initial DAR
2004/April	Addendum 1
2004/August	Addendum revised 1
2005/January	Addendum revised 2
2005/April	Addendum Ecotox
2018/May	dRAR
2018/July	dRAR revised 1

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B.9. ECOTOXICOLOGY DATA

Triticonazole is a fungicidal active substance and was included into Annex I of Directive 91/414/EEC in 2005 (Directive 2006/39/EC, 12th April 2006). Directive 91/414/EEC has been repealed by Regulation (EC) no 1107/2009 of 21 October 2009 concerning the placing of plant protection products on the market. Accordingly triticonazole is deemed to have been approved under Regulation (EC) no 1107/2009, as set out in Part A of the Annex of Commission Implementing Regulation (EC) no 540/2011 as regards the list of approved substances (entry no. 127).

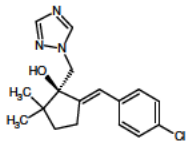
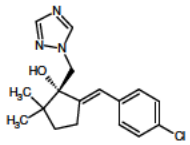
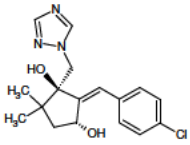
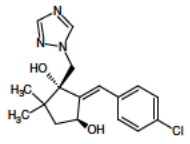
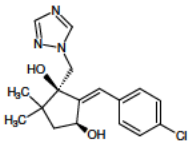
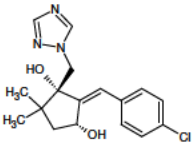
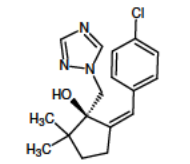
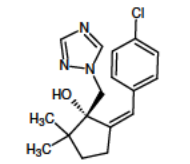
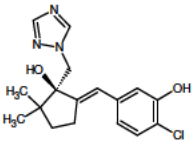
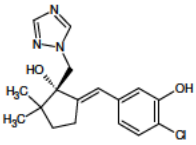
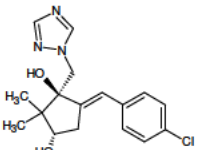
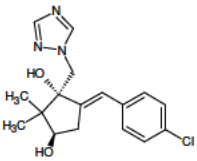
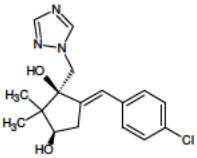
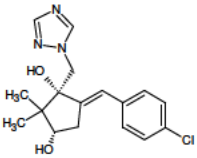
This draft renewal assessment report (dRAR) contains summaries of studies on triticonazole, which were not available at the time of the Annex I inclusion under Directive 91/414/EEC and were, therefore, not evaluated during the first EU review of this compound. In addition, studies, which were already submitted for the Annex I inclusion under Directive 91/414/EEC, were re-evaluated according to the currently valid test guidelines and were summarised in the dRAR (study title is greyed out).
Studies which were submitted for the first EU peer-review of the active substance triticonazole but are no longer a data requirement according to the data requirements for active substances (Commission Regulation (EU) 283/2013) and/or plant protection products (Commission Regulation (EU) 284/2013) are briefly summarised (text in *italic*).

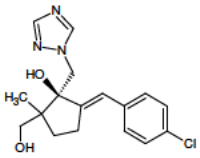
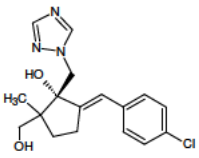
In case where reliable and adequate literature was found during the literature search, summaries are integrated in the respective sections of the dRAR.

Triticonazole is a racemic mixture of two enantiomers. The fungicidal activity of the two enantiomers has been shown to be equivalent and not different from the racemic mixture. In degradation studies (non-guideline lysimeter study and in a water sediment study) no significant changes in the ratio of the racemate (1:1) were observed, indicating that the degradation and distribution of both enantiomers is the same in the environment. Therefore it was considered adequate that all studies on the active substance where performed using the racemic mixture.

The different synonyms and codes for the active substance triticonazole and its metabolites used in the dRAR are summarised in the Table 9-1.

Table 9-1: Substances and metabolites (structure, synonyms and codes)

Code number (Synonyms)	Chemical name and molecular formula	Mol weight (g/mol)	Occurrence (% AR)	Structure
Triticonazole E-isomer (BAS 595 F, RPA 400727, M595F000)	(1*RS*, 5*E*)-5-(4-chlorobenzylidene)-2,2-dimethyl-1-(1*H*-1,2,4-triazol-1-ylmethyl) cyclopentanol $C_{17}H_{20}ClN_3O$	317.8	Not applicable	 *R*-isomer  *S*-isomer
RPA 406341 Trans-diol (M595F002, AE 0540093, Reg. No. 5059144)	(1*RS*,2*E*,3*SR*)-2-(4-chlorobenzylidene)-5,5-dimethyl-1-(1*H*-1,2,4-triazol-1-ylmethyl)-1,3-cyclopentanediol $C_{17}H_{20}ClN_3O_2$	333.8	**Aerobic soil: 20.2** Anaerobic soil: 1.8 Soil photolysis: 3.5 Aquatic hydrolysis: ni Aquatic photolysis: ni Aerobic surface water: 1.8 Water/sediment: ni	 *RS*-isomer  *SR*-isomer
RPA 404766 Cis-diol (M595F001, AE 0591653, Reg. No. 5079285)	(1*RS*,2*E*,3*RS*)-2-(4-chlorobenzylidene)-5,5-dimethyl-1-(1*H*-1,2,4-triazol-1-ylmethyl)-1,3-cyclopentanediol $C_{17}H_{20}ClN_3O_2$	333.8	**Aerobic soil: 13.9**[(a)] Anaerobic soil: 2.0 Soil photolysis: 3.3 Aquatic hydrolysis: ni Aquatic photolysis: ns Aerobic surface water: 1.3 Water/sediment: ni	 *RR*-isomer  *SS*-isomer
RPA 406203 Z-isomer (M595F014, Reg. No. 5079359)	(1*RS*, 5*Z*)-5-(4-chlorobenzylidene)-2,2-dimethyl-1-(1*H*-1,2,4-triazol-1-ylmethyl) cyclopentanol $C_{17}H_{20}ClN_3O$	317.8	Aerobic soil: 4.4 Anaerobic soil: - **Soil photolysis: 11.0** Aquatic hydrolysis: 2.6 **Aquatic photolysis: 42.3**[(b)] Aerobic surface water: 4.2[(c)] Water/sediment: ni	 *R*-isomer  *S*-isomer
RPA 407922 (M595F013, Reg. No. 5079288)	2-chloro-5-{(E)-[(2RS)-2-hydroxy-3,3-dimethyl-2-(1H-1,2,4-triazol-1-ylmethyl)cyclopentylidene]methyl}phenol $C_{17}H_{20}ClN_3O_2$	333.8	Unlikely to occur at significant (> 5 % AR) amounts in environmental compartments	 *R*-isomer  *S*-isomer
RPA 406780[(d)]	(1SR,3RS,5E)-5-(4-chlorobenzylidene)-2,2-dimethyl-1-(1H-1,2,4-triazol-1-ylmethyl)cyclopentane-1,3-diol $C_{17}H_{20}ClN_3O_2$	333.8	Unlikely to occur at significant (> 5 % AR) amounts in environmental compartments	 *RS*-isomer  *SR*-isomer  *RR*-isomer  *SS*-isomer

Code number (Synonyms)	Chemical name and molecular formula	Mol weight (g/mol)	Occurrence (% AR)	Structure
RPA 404886	(1*RS*,5*E*)-5-(4-chlorobenzylidene)-2-(hydroxymethyl)-2-methyl-1-(1*H*-1,2,4-triazol-1-ylmethyl) cyclopentanol $C_{17}H_{20}ClN_3O_2$	333.8	Unlikely to occur at significant (> 5 % AR) amounts in environmental compartments	 *R*-isomer  *S*-isomer

ni denotes not investigated (below 5 % AR)
ns denotes not stated (below 5 % AR)
(a) 10 °C study (max. 9.9 % AR in 20 - 25 °C studies)
(b) Without sensitizer
(c) Arithmetic mean of phenyl and triazole label
(d) Isomeric composition not specified further (Trans- vs. Cis-diol)

B.9.1. EFFECTS ON BIRDS AND OTHER TERRESTRIAL VERTEBRATES

B.9.1.1. Effects on birds

One additional one-generation bird toxicity study ([redacted], 2012a) was performed, which was not submitted during the first EU peer-review process. The studies submitted for the first EU approval of the active substance are summarised below. In addition, the studies were evaluated according to the representative test guidelines.

B.9.1.1.1. Acute oral toxicity to Birds

Active substance:

Reference:	**RPA 400727: Acute Oral Toxicity (LD_{50}) to Bobwhite Quail**
Author(s), year:	[redacted] 1991a
Report/Doc. number:	Document No. R013025
Guideline(s):	US EPA Subdivision E, Section 71-1 (Avian single-dose oral LD_{50}), October 1982
GLP:	Yes
Deviations:	Please refer to the commenting box below
Validity:	Acceptable

Material and methods:

Test substance:	RPA 400727, batch : DA 640, purity : 95.9%
Test species:	Bobwhite quail (*Colinus virginianus*)
Number of organisms:	5 males and 5 females per treatment group
Weight, age:	181 – 244 g bodyweight, approximately 22 weeks old
Type of test:	Acute oral toxicity
Range finding test:	1 male and 1 female from HRC stock dosed at 2000 mg ai/kg bw
Applied concentrations:	0 (1% methylcellulose vehicle only), 500, 1000 and 2000 mg ai/kg bw, dosage volume: dosage volume: 10 mL/kg body weight
Type of application:	Oral intubation using a Ch 10 plastic catheter
Time of exposure:	One single application, monitoring during 14 days
Test conditions:	Test temperature: mean daily minimum - 21°C mean daily maximum - 23°C, relative humidity: mean daily – 85%, lighting: 10h light and 14h darkness. Feed (standard HRC layer diet) in pellet form was provided ad libitum during acclimation and during the test, except of an overnight starvation period of approx. 19 hours prior to testing.
Acclimation:	15 days prior to dosing

Test parameter:

Observations:	Mortalities, bird health and clinical signs were observed daily. Individual

bodyweights were observed on Days -15, -7 (pre-treatment period) and 0 (immediately prior to dosing), and on Days 7 and 14 (post-treatment period). Group mean food consumption was measured weekly over the following periods: Days -15 to -8, -7 to -1, 1 to 7 and 8 to 14.

At test termination, post-mortem examination was carried out on ten birds from the high dose group.

Statistics: There were no mortalities in this study. Therefore, it was not possible to perform a calculation of $LD_{50,}$ LD_{20} and LD_{10} values. The LD_{50} value was determined to be greater than the highest dosage tested. Analysis of variance (Williams 1971, 1972), were carried out for bodyweights on Day 7 and Day 14 for all groups.

Findings:

Mortalities: No mortalities occurred in the control group and in the 500, 1000 and 2000 mg ai/kg treatment groups.

Clinical signs: All control birds and all birds in the treatment groups were normal in appearance and behaviour throughout the test.

Body weight, feed consumption: Compared to the control group, there were no treatment-related effects on body weight and feed consumption in the treatment groups.

Table 9.1-1: Mortality and growth (bodyweights and food consumption) of bobwhite quail following acute oral exposure (gavage)

Test substance [mg ai/kg]	Cumulative mortality	Sex	Mean bodyweight change [g/bird]				Mean food consumption [g/bird/d]			
			-15 to -7	-7 to 0	0 to 7	7 to 14	-15 to -8	-7 to -1	1 to 7	8 to 14
Control	0	♂	0	-7	+4	+1	14	13	15	16
	0	♀	+2	-14	-4	-1	20	18	16	17
500	0	♂	0	-6	-1	+2	15	14	13	17
	0	♀	0	-12	-5	-2	19	18	15	16
1000	0	♂	0	-5	+6	+5	15	13	14	17
	0	♀	-9	-15	+3	0	15	16	14	17
2000	0	♂	-2	-4	+4	+4	13	12	11	15
	0	♀	-8	-13	-2	+2	18	12	12	16

Conclusion: LD_{50} > 2000 mg ai/kg bw, NOEL = 2000 mg ai/kg bw

Comment RMS: **Validity:** The study was evaluated according to the current valid test guidelines OCSPP 850.2100 (2012) and OECD 223 (2010).

Check of validity criteria:

OCSPP 850.2100 (2012):

- Birds were randomly assigned to treatment and control pens. Fulfilled.
- Not more than 10% of the control birds died during the test. The control in the

current study was 0%. Fulfilled.

- At least ten birds were used for each dose level of the test substance and control. Fulfilled.

– The test substance was orally administered, via either capsule or gavage. Fulfilled.

- A minimum of five dose levels of the test substance, plus an appropriate control were tested. In the current study three concentrations were tested.

OECD 223 (2010):

- The mortality in the controls should not exceed 10 per cent. In the current study the mortality was 0%. Fulfilled.

In addition, the following points deviated from the test guideline or were not reported in detail:

- According to OECD 223 the photoperiod for quail and mallard should be eight hours light and 16 hours dark. In the current study the photoperiod was 10 hours light and 14 hours dark as recommended in OCSPP 850.2100.

Acceptability of the analytical methods used in the test: No analytical methods were reported.

Endpoints:

The RMS agrees on the endpoints given in the study report.

LD_{50} is > 2000 mg ai/kg bw

No LD_{10} or LD_{20} values could be determined due to the lack of mortality.

Conclusion of the RMS: Only three concentrations were tested. However, as the LD_{50} is > 2000 mg ai/kg bw, the test can be assumed to be a limit test. Based on the evaluation of the study the bird acute toxicity test is considered valid.

Reference:	**RPA 400727: Acute Oral Toxicity (LD_{50}) to Mallard Duck**
Author(s), year:	[redacted] 1991b
Report/Doc. number:	Document No. R013024
Guideline(s):	US EPA Subdivision E, Section 71-1 (Avian single-dose oral LD_{50}), October 1982
GLP:	Yes
Deviations:	Please refer to the commenting box below
Validity:	Acceptable

Material and methods:

Test substance:	RPA 400727, batch : DA 640, purity : 95.9%
Test species:	Mallard Duck (*Anas platyrhynchos*)
Number of organisms:	5 males and 5 females per treatment group

Weight, age:	905 - 1185 g bodyweight, approximately 21 month old
Type of test:	Acute oral toxicity
Range finding test:	1 male and 1 female from HRC stock dosed at 2000 mg ai/kg bw – emetic reaction recorded
Applied concentrations:	0 (1% methylcellulose vehicle only), 125, 250, 500, 1000 and 2000 mg ai/kg bw, dosage volume: dosage volume: 5 mL/kg body weight
Type of application:	Oral intubation using a Ch 14 plastic catheter,
Time of exposure:	One single application, monitoring during 14 days
Test conditions:	Test temperature: mean daily minimum - 17°C mean daily maximum - 22°C, relative humidity: mean daily – 91%, lighting: 10h light and 14h darkness. Feed (standard HRC layer diet) in pellet form was provided ad libitum during acclimation and during the test, except of an overnight starvation period of approx. 19 hours prior to testing.
Acclimation:	15 days prior to dosing
<u>Test parameter:</u>	
Observations:	Mortalities, bird health and clinical signs were observed daily. Individual bodyweights were observed on Days -15, -7 (pre-treatment period) and 0 (immediately prior to dosing), and on Days 7 and 14 (post-treatment period). Group mean food consumption was measured weekly over the following periods: Days -15 to -8, -7 to -1, 1 to 7 and 8 to 14. At test termination, post-mortem examination was carried out on ten birds from the high dose group.
Statistics:	There were no mortalities in this study. Therefore, it was not possible to perform a calculation of a LD_{50}. The LD_{50} value was determined to be greater than the highest dosage tested. Analysis of variance (Williams 1971, 1972), were carried out for bodyweights on Day 7 and Day 14 for all groups.
<u>Findings:</u>	
Mortalities:	No mortalities occurred in the control group and in the 500, 1000 and 2000 mg ai/kg treatment groups.
Clinical signs:	All control birds were normal in appearance and behaviour throughout the test. Five birds in one group vomited dose-like material shortly after dosing. All other birds remained in good health.
Body weight, feed consumption:	One bird was subdued on Day -1 and replaced at time of dosing with a new bird. Compared to the control group, there were no treatment-related effects on body weight and feed consumption in the treatment groups.

Table 9.1-2: Mortality and growth (bodyweights and food consumption) of mallard duck following acute oral exposure (gavage)

Test substance [mg ai/kg]	Cumulative mortality	Sex	Mean bodyweight change [g/bird]				Mean food consumption [g/bird/d]			
			-15 to -7	-7 to 0	0 to 7	7 to 14	-15 to -8	-7 to -1	1 to 7	8 to 14
Control	0	♂	+10	-46	+71	+12	65	80	111	111
	0	♀	+19	-39	+50	+34	65	114	120	126
125	0	♂	+1	-66	+66	+9	53	60	71	69
	0	♀	-34	-15	+57	+2	48	97	120	131
250	0	♂	+27	-54	+62	-2	90	126	126	114
	0	♀	-30	-46	+44	+15	38	69	69	83
500	0	♂	+18	-64	+68	-31	75	74	129	140
	0	♀	-32	-53	+49	-13	48	69	89	80
1000	0	♂	+11	-53	+78	-8	63	63	91	86
	0	♀	-19	-48	+33	+4	43	51	63	74
2000	0	♂	+9	-66	+43	-1	55	57	74	74
	0	♀	-51	-61	+65	+24	40	49	86	74

Conclusion: LD_{50} > 2000 mg ai/kg bw, NOEL = 1000 mg ai/kg bw (based upon vomiting incidents)

Comment RMS:

Validity: The study was evaluated according to the current valid test guidelines OCSPP 850.2100 (2012) and OECD 223 (2010).

Check of validity criteria:

OCSPP 850.2100 (2012):

- Birds were randomly assigned to treatment and control pens. Fulfilled.
- Not more than 10% of the control birds died during the test. The control in the current study was 0%. Fulfilled.
- At least ten birds were used for each dose level of the test substance and control. Fulfilled.
– The test substance was orally administered, via either capsule or gavage. Fulfilled.
- A minimum of five dose levels of the test substance, plus an appropriate control were tested. Fulfilled.

OECD 223 (2010):

- The mortality in the controls should not exceed 10 per cent. In the current study the mortality was 0%. Fulfilled.

In addition, the following points deviated from the test guideline or were not reported in detail:

- According to OECD 223 the photoperiod for quail and mallard should be eight hours light and 16 hours dark. In the current study the photoperiod was 10 hours

	light and 14 hours dark as recommended in OCSPP 850.2100. **Acceptability of the analytical methods used in the test:** No analytical methods were reported. **Endpoints:** The RMS agrees on the endpoints given in the study report. LD_{50} is > 2000 mg ai/kg bw NOEL = 1000 mg ai/kg bw (based upon vomiting incidents) No LD_{10} or LD_{20} values could be determined due to the lack of mortality. **Conclusion of the RMS:** Based on the evaluation of the study the bird acute toxicity test is considered valid.

Reference:	**RPA 400727: Acute Oral Toxicity (LD_{50}) to Grey Partridge**
Author(s), year:	[redacted] 1992a
Report/Doc. number:	Document No. R013069
Guideline(s):	US EPA Subdivision E, Section 71-1 (Avian single-dose oral LD_{50}), October 1982
GLP:	Yes
Deviations:	Please refer to the commenting box below
Validity:	Acceptable

Material and methods:

Test substance:	RPA 400727, batch : DA 640, purity : 95.9%
Test species:	Grey Partridge (*Perdix perdix*)
Number of organisms:	5 males and 5 females per treatment group
Weight, age:	333 - 440 g bodyweight, > 9 month old
Type of test:	Acute oral toxicity
Range finding test:	1 male and 1 female from HRC stock dosed at 2000 mg ai/kg bw
Applied concentrations:	0 (1% methylcellulose vehicle only), 500, 1000 and 2000 mg ai/kg bw, dosage volume: dosage volume: 10 mL/kg body weight
Type of application:	Oral intubation using a Ch 14 plastic catheter,
Time of exposure:	One single application, monitoring during 14 days
Test conditions:	Test temperature: mean daily minimum - 17°C mean daily maximum - 19°C, relative humidity: mean daily – 76%, lighting: 7h light and 17h darkness. Feed (standard HRC layer diet) in pellet form was provided ad libitum during acclimation and during the test, except of an overnight starvation period of approx. 19 hours prior to testing.
Acclimation:	15 days prior to dosing

Test parameter:

Observations:	Mortalities, bird health and clinical signs were observed daily. Individual

bodyweights were observed on Days -15, -7 (pre-treatment period) and 0 (immediately prior to dosing), and on Days 7 and 14 (post-treatment period). Group mean food consumption was measured weekly over the following periods: Days -15 to -8, -7 to -1, 1 to 7 and 8 to 14.

At test termination, post-mortem examination was carried out on ten birds from the high dose group.

Statistics: There were no mortalities in this study. Therefore, it was not possible to perform a calculation of a LD_{50}. The LD_{50} value was determined to be greater than the highest dosage tested. Analysis of variance (Williams 1971, 1972), were carried out for bodyweights on Day 7 and Day 14 for all groups.

Findings:

Mortalities: No mortalities occurred in the control group and in the 500, 1000 and 2000 mg ai/kg treatment groups.

Clinical signs: All control birds and all birds in the treatment groups were normal in appearance and behaviour throughout the test.

Body weight, feed consumption: Compared to the control group, there were no treatment-related effects on body weight and feed consumption in the treatment groups.

Table 9.1-3: Mortality and growth (bodyweights and food consumption) of grey partridge following acute oral exposure (gavage)

Test substance [mg ai/kg]	Cumulative mortality	Sex	Mean bodyweight change [g/bird]				Mean food consumption [g/bird/d]			
			-15 to -7	-7 to 0	0 to 7	7 to 14	-15 to -8	-7 to -1	1 to 7	8 to 14
Control	0	♂	-2	-6	+7	+1	18	23	29	17
	0	♀	-6	-2	+3	+3	25	20	26	14
500	0	♂	-5	-11	+11	+2	20	20	29	26
	0	♀	+1	-3	+11	+2	18	11	37	14
1000	0	♂	+2	-3	+3	+3	15	40	23	20
	0	♀	-5	-9	+15	+4	18	26	23	17
2000	0	♂	-3	-4	0	0	20	23	20	23
	0	♀	-6	-7	-2	+7	15	23	20	20

Conclusion: LD_{50} > 2000 mg ai/kg bw, NOEL = 2000 mg ai/kg bw

Comment RMS: **Validity:** The study was evaluated according to the current valid test guidelines OCSPP 850.2100 (2012) and OECD 223 (2010).

Check of validity criteria:

OCSPP 850.2100 (2012):

- Birds were randomly assigned to treatment and control pens. Fulfilled.
- Not more than 10% of the control birds died during the test. The control in the

current study was 0%. Fulfilled.

- At least ten birds were used for each dose level of the test substance and control. Fulfilled.

– The test substance was orally administered, via either capsule or gavage. Fulfilled.

- A minimum of five dose levels of the test substance, plus an appropriate control were tested. In the current study three concentrations were tested.

OECD 223 (2010):

- The mortality in the controls should not exceed 10 per cent. In the current study the mortality was 0%. Fulfilled.

In addition, the following points deviated from the test guideline or were not reported in detail:

- According to OCSPP 850.2100 relative humidity should be maintained between 45 and 70%. In the current study the mean daily relative humidity was 76%.

- According to OECD 223 the photoperiod for quail and mallard should be eight hours light and 16 hours dark, according OCSPP 850.2100 it should be 10 hours light and 14 hours dark. In the current study the photoperiod was 7 hours light and 17 hours dark.

- It is noted that the grey partridge is not listed among the recommended test species in the current guidelines.

Acceptability of the analytical methods used in the test: No analytical methods were reported.

Endpoints:

The RMS agrees on the endpoints given in the study report.

LD_{50} is > 2000 mg ai/kg bw

NOEL = 2000 mg ai/kg bw

No LD_{10} or LD_{20} values could be determined due to the lack of mortality.

Conclusion of the RMS: Only three concentrations were tested. However, as the LD_{50} is > 2000 mg ai/kg bw, the test can be assumed to be a limit test. Based on the evaluation of the study the bird acute toxicity test is considered valid.

Reference:	**RPA 400727: Acute Oral Toxicity (LD_{50}) to Red-legged Partridge**
Author(s), year:	[redacted] 1992b
Report/Doc. number:	Document No. R013065
Guideline(s):	US EPA Subdivision E, Section 71-1 (Avian single-dose oral LD_{50}), October 1982
GLP:	Yes
Deviations:	Please refer to the commenting box below
Validity:	Acceptable

Material and methods:

Test substance:	RPA 400727, batch : DA 640, purity : 95.9%
Test species:	Red-legged Partridge (*Alectoris rufa*)
Number of organisms:	5 males and 5 females per treatment group
Weight, age:	378 - 584 g bodyweight, > 7 month old
Type of test:	Acute oral toxicity
Range finding test:	1 male and 1 female birds from HRC stock dosed at 2000 mg ai/kg bw
Applied concentrations:	0 (1% methylcellulose vehicle only), 500, 1000 and 2000 mg ai/kg bw, dosage volume: dosage volume: 10 mL/kg body weight
Type of application:	Oral intubation using a Ch 14 plastic catheter
Time of exposure:	One single application, monitoring during 14 days
Test conditions:	Test temperature: mean daily minimum - 19°C mean daily maximum - 21°C, relative humidity: mean daily – 78%, lighting: 7h light and 17h darkness. Feed (standard HRC layer diet) in pellet form was provided ad libitum during acclimation and during the test, except of an overnight starvation period of approx. 19 hours prior to testing.
Acclimation:	15 days prior to dosing

Test parameter:

Observations:	Mortalities, bird health and clinical signs were observed daily. Individual bodyweights were observed on Days -15, -7 (pre-treatment period) and 0 (immediately prior to dosing), and on Days 7 and 14 (post-treatment period). Group mean food consumption was measured weekly over the following periods: Days -15 to -8, -7 to -1, 1 to 7 and 8 to 14. At test termination, post-mortem examination was carried out on ten birds from the highest dose and 10 birds from the controls.
Statistics:	There were no mortalities in this study. Therefore, it was not possible to perform a calculation of a LD_{50}. The LD_{50} value was determined to be greater than the highest dosage tested. Analysis of variance (Williams 1971, 1972), were carried out for bodyweights on Day 7 and Day 14 for all groups.

Findings:

Mortalities:	No mortalities occurred in the control group and in the 500, 1000 and 2000 mg

ai/kg treatment groups.

Clinical signs: All control birds and all birds in the treatment groups were normal in appearance and behaviour throughout the test.

Body weight, feed consumption: On Day 14, all three male treated groups had significantly lower bodyweights when compared with the control ($P<0.05$). The female high dose group had significantly lower bodyweights, on Day 7, when compared with the control ($P<0.05$). However, there was no dose-response relation in male data. However, no dose-response relationship was seen in the results.

Table 9.1-4: Mortality and growth (bodyweights and food consumption) of red-legged partridge following acute oral exposure (gavage)

Test substance [mg ai/kg]	Cumulative mortality	Sex	Mean bodyweight change [g/bird]				Mean food consumption [g/bird/d]			
			-15 to -7	-7 to 0	0 to 7	7 to 14	-15 to -8	-7 to -1	1 to 7	8 to 14
Control	0	♂	-2	-3	+16	+7	18	26	26	23
	0	♀	-3	-4	+10	+2	20	14	34	17
500	0	♂	+18	-7	+6	-13	20	23	20	20
	0	♀	+4	-7	+7	+1	15	23	17	20
1000	0	♂	0	-7	+9	+1	20	17	17	20
	0	♀	+2	-7	+8	+3	33	17	20	11
2000	0	♂	+1	+14	-10	+6	23	23	31	26
	0	♀	+1	-7	+3	+6	20	14	17	20

Conclusion: LD_{50} > 2000 mg ai/kg bw, NOEL could not be determined due to bodyweight changes

Comment RMS:

Validity: The study was evaluated according to the current valid test guidelines OCSPP 850.2100 (2012) and OECD 223 (2010).

Check of validity criteria:

OCSPP 850.2100 (2012):

- Birds were randomly assigned to treatment and control pens. Fulfilled.
- Not more than 10% of the control birds died during the test. The control in the current study was 0%. Fulfilled.
- At least ten birds were used for each dose level of the test substance and control. Fulfilled.
– The test substance was orally administered, via either capsule or gavage. Fulfilled.
- A minimum of five dose levels of the test substance, plus an appropriate control were tested. In the current study three concentrations were tested.

OECD 223 (2010):

- The mortality in the controls should not exceed 10 per cent. In the current study

	the mortality was 0%. Fulfilled. **In addition, the following points deviated from the test guideline or were not reported in detail:** - According to OCSPP 850.2100 relative humidity should be maintained between 45 and 70%. In the current study the mean daily relative humidity was 78%. - According to OECD 223 the photoperiod for quail and mallard should be eight hours light and 16 hours dark, according OCSPP 850.2100 it should be 10 hours light and 14 hours dark. In the current study the photoperiod was 7 hours light and 17 hours dark. - It is noted that the red-legged partridge is not listed among the recommended test species in the OECD 223. **Acceptability of the analytical methods used in the test:** No analytical methods were reported. **Endpoints:** The RMS agrees on the endpoints given in the study report. LD_{50} is > 2000 mg ai/kg bw **Conclusion of the RMS:** Only three concentrations were tested. However, as the LD_{50} is > 2000 mg ai/kg bw, the test can be assumed to be a limit test. Based on the evaluation of the study the bird acute toxicity test is considered valid.

Reference:	**The Acute Oral Toxicity of RPA 400727 to the Pigeon**
Author(s), year:	[redacted], M., 1990a
Report/Doc. number:	Document No. R013005
Guideline(s):	-
GLP:	No
Deviations:	Please refer to the commenting box below
Validity:	Should only be used as additional information

Material and methods:

Test substance:	RPA 400727, batch : BD 1036 D, purity : 98 - 100%
Test species:	Domestic Pigeon (*Columba livia*)
Number of organisms:	2 males and 2females per treatment group
Weight, age:	340 - 492 g bodyweight, adult
Type of test:	Acute oral toxicity
Range finding test:	2 male and 2 female birds from HRC stock dosed at 1000 and 2000 mg ai/kg bw, respectively. Regurgitation 20 minutes after dosing in the 2000 mg testing group and 40 minutes after dosing in the 1000 mg testing group. Both birds from the 1000 mg testing group were subdued 3.5 hours after dosing but had recovered 18

	hours later.
Applied concentrations:	0 (corn oil vehicle only), 125, 250, 500, 1000 and 2000 mg ai/kg bw, dosage volume: dosage volume: 10 mL/kg body weight
Type of application:	Oral intubation using a Ch 14 plastic catheter
Time of exposure:	One single application, monitoring during 14 days
Test conditions:	Test temperature: mean daily minimum - 19°C mean daily maximum - 21°C, relative humidity: mean daily – 52%, lighting: 7h light and 17h darkness, 120 lux Birds were given weed grain ad libitum, except of an overnight starvation of at least 15 hours period prior to testing
Acclimation:	14 days prior to dosing
Test parameter:	
Observations:	Mortalities, bird health and clinical signs were observed daily. Individual bodyweights were observed on Days -14, -7 (pre-treatment period) and 0 (immediately prior to dosing), and on Days 7 and 14 (post-treatment period). Group mean food consumption was measured weekly over the following periods: Days -14 to -8, -7 to -1, 1 to 7 and 8 to 14. At test termination, post-mortem examination was carried out on all birds from the highest dose
Statistics:	There were no mortalities in this study. Therefore, it was not possible to perform a calculation of a LD_{50}. The LD_{50} value was determined to be greater than the highest dosage tested.
Findings:	
Mortalities:	No mortalities occurred in the control group and in all treatment groups.
Clinical signs:	Regurgitation of feed or dose occurred at 500, 1000 and 2000 mg ai/kg bw.
Body weight, feed consumption:	Bodyweight changes were similar in all groups with no evidence of any treatment-related effect.

Table 9.1-5: Mortality and growth (bodyweights and food consumption) of pigeon following acute oral exposure (gavage)

Test substance [mg ai/kg]	Cumulative mortality	Sex	Mean bodyweight change [g/bird]			
			-14to -7	-7 to 0	0 to 7	7 to 14
Control	0	♂	+44	-7	+7	+11
	0	♀	+9	-2	+6	+1
125	0	♂	+11	-37	+35	-1
	0	♀	+40	+12	-9	+8
250	0	♂	+49	-11	+18	-4
	0	♀	+18	-10	+4	+9
500	0	♂	+17	-5	+3	+7
	0	♀	+17	-6	+5	+1

Test substance [mg ai/kg]	Cumulative mortality	Sex	Mean bodyweight change [g/bird]			
			-14to -7	-7 to 0	0 to 7	7 to 14
1000	0	♂	-15	-6	+1	+11
	0	♀	+17	-4	+7	+3
2000	0	♂	+26	-2	+2	+2
	0	♀	+36	-6	+5	0

Conclusion: LD_{50} > 2000 mg ai/kg bw, NOEL = 250 mg ai/kg bw (non-emetic)

Comment RMS:

Validity: The study was evaluated according to the current valid test guidelines OCSPP 850.2100 (2012) and OECD 223 (2010).

Check of validity criteria:

OCSPP 850.2100 (2012):

- Birds were randomly assigned to treatment and control pens. Fulfilled.
- Not more than 10% of the control birds died during the test. The control in the current study was 0%. Fulfilled.
- At least ten birds were used for each dose level of the test substance and control. In the current study two female and two male birds were used per treatment group. At the end of the study only birds in treatment group 2000 mg ai/kg were subjected to necropsy.
– The test substance was orally administered, via either capsule or gavage. Fulfilled.
- A minimum of five dose levels of the test substance, plus an appropriate control were tested. Fulfilled.

OECD 223 (2010):

- The mortality in the controls should not exceed 10 per cent. In the current study the mortality was 0%. Fulfilled.

In addition, the following points deviated from the test guideline or were not reported in detail:

- According to OECD 223 the photoperiod for quail and mallard should be eight hours light and 16 hours dark, according OCSPP 850.2100 it should be 10 hours light and 14 hours dark. In the current study the photoperiod was 7 hours light and 17 hours dark.
- It is noted that the pigeon is not listed among the recommended test species in the OECD 223.

Acceptability of the analytical methods used in the test: No analytical methods were reported.

Endpoints:

	The RMS agrees on the endpoints given in the study report. LD_{50} is > 2000 mg ai/kg bw NOEL = 250 mg ai/kg bw (non-emetic) No LD_{10} or LD_{20} values could be determined due to the lack of mortality. **Conclusion of the RMS:** As a screening test the study can be used as additional information.

Reference:	**The Acute Oral Toxicity of RPA 400727 to Ring-necked Pheasant**
Author(s), year:	[redacted] 1990b
Report/Doc. number:	Document No. R013004
Guideline(s):	-
GLP:	No
Deviations:	Please refert to the commenting box below
Validity:	Should only be used as additional information

Material and methods:

Test substance:	RPA 400-727, batch : BD 1037 and BD 1036D, purity : 98-100%
Test species:	Ring-necked pheasant (*Phasianus colchicus*)
Number of organisms:	2 males and 2 females per treatment group
Weight, age:	925 – 1475 g bodyweight, adult
Type of test:	Acute oral toxicity
Range finding test:	1 male and 1 female dosed at 2000 mg ai/kg bw
Applied concentrations:	0 (corn oil only), 500, 1000 and 2000 mg ai/kg bw, dosage volume: dosage volume: 10 mL/kg body weight
Type of application:	Oral intubation using a Ch 14 plastic catheter
Time of exposure:	One single application, monitoring during 14 days
Test conditions:	Test temperature: mean daily minimum - 15°C (SD = 3) mean daily maximum - 17°C (SD = 3), relative humidity: mean daily – 66% (SD = 10.2), lighting: 7h light and 17h darkness; 10 lux. Feed (standard HRC layer diet) in pellet form was provided ad libitum during acclimation and during the test, except of an overnight starvation period of approx. 15 hours.
Acclimation:	14 days prior to dosing

Test parameter:

Observations:	Mortalities, bird health and clinical signs were observed daily. Individual bodyweights were observed on Days -14, -7 (pre-treatment period) and 0 (immediately prior to dosing), and on Days 7 and 14. At test termination, post-mortem examination was carried out on four birds from the highest surviving dose

group.

Statistics: There were no mortalities in this study. Therefore, it was not possible to perform a calculation of a LD_{50}. The LD_{50} value was determined to be greater than the highest dosage tested.

Findings:

Mortalities: One bird died on Day -4 and was replaced by a spare bird. No mortalities occurred in the control group and in the 500, 1000 and 2000 mg ai/kg treatment groups.

Clinical signs: All control birds and all birds in the treatment groups were normal in appearance and behaviour throughout the test.

Body weight, feed consumption: Bodyweight changes were variable with no clear evidence of a treatment-related effect.

Table 9.1-6: Mortality and growth (bodyweights and food consumption) of ring-necked pheasant following acute oral exposure (gavage)

Test substance [mg ai/kg]	Cumulative mortality	Sex	Mean bodyweight change [g/bird]			
			-14 to -7	-7 to 0	0 to 7	7 to 14
Control	0	♂	-25	-45	+20	+42
	0	♀	+5	+10	-35	+20
500	0	♂	+13	-40	+10	-28
	0	♀	+52	-7	-28	+35
1000	0	♂	+10	-3	-25	+63
	0	♀	+25	+2	-52	+27
2000	0	♂	-48	-2	-28	+28
	0	♀	-7	-8	-45	+30

Conclusion: LD_{50} > 2000 mg ai/kg bw, NOEL = 2000 mg ai/kg bw

Comment RMS:

Validity: The study was evaluated according to the current valid test guidelines OPCSPP 850.2100 (2012) and OECD 223 (2010).

Check of validity criteria:

OCSPP 850.2100 (2012):

- Birds were randomly assigned to treatment and control pens. Fulfilled.
- Not more than 10% of the control birds died during the test. The control in the current study was 0%. Fulfilled.
- At least ten birds were used for each dose level of the test substance and control. In the current study two female and two male birds were used per treatment group. At the end of the study only birds in treatment group 2000 mg ai/kg were subjected to necropsy.
– The test substance was orally administered, via either capsule or gavage.

	Fulfilled. - A minimum of five dose levels of the test substance, plus an appropriate control were tested. In the current study three concentrations were tested OECD 223 (2010): - The mortality in the controls should not exceed 10 per cent. In the current study the mortality was 0%. Fulfilled. **In addition, the following points deviated from the test guideline or were not reported in detail:** - According to OECD 223 the photoperiod for quail and mallard should be eight hours light and 16 hours dark, according OCSPP 850.2100 it should be 10 hours light and 14 hours dark. In the current study the photoperiod was 7 hours light and 17 hours dark. - It is noted that the ring-necked pheasant is not listed among the recommended test species in the OECD 223. **Acceptability of the analytical methods used in the test:** No analytical methods were reported. **Endpoints:** The RMS agrees on the endpoints given in the study report. LD_{50} is > 2000 mg ai/kg bw NOEL = 2000 mg ai/kg bw No LD_{10} or LD_{20} values could be determined due to the lack of mortality. **Conclusion of the RMS:** only three concentrations were tested. However, as the LD_{50} is > 2000 mg ai/kg bw, the test can be assumed to be a limit test. As a screening test the study can be used as additional information.

Metabolites:

Reference:	**RPA 406341: An Acute Oral Toxicity Study with the Northern Bobwhite**
Author(s), year:	[redacted] 2000a
Report/Doc. number:	Document No. B002787
Guideline(s):	US EPA guideline OPPTS 850.2100 (April 1996) and US EPA Subdivision E, Section 71-1 (Avian single-dose oral LD_{50}), October 1982
GLP:	Yes
Deviations:	Please refer to the commenting box below
Validity:	Acceptable

Material and methods:

Test substance:	RPA 406341, batch : BESS05996, purity : 100%
Test species:	Northern Bobwhite (*Colinus virginianus*)

Number of organisms:	5 males and 5 females per treatment group
Weight, age:	169- 213 g bodyweight, 18 weeks
Type of test:	Acute oral toxicity
Range finding test:	The dosages were established based on toxicity data provided by the sponsor.
Applied concentrations:	0 (corn oil vehicle only), 295, 486, 810, 1350 and 2250 mg/kg bw, dosage volume: dosage volume: 5 mL/kg body weight
Type of application:	Oral intubation using stainless steel 14 gauge canula
Time of exposure:	One single application, monitoring during 14 days
Test conditions:	Average test temperature: 23.8 ± 1.2°C (SD), average relative humidity: 60% ± 13% (SD), lighting: 8h light and 16h darkness with approx. 128 lux. Feed of a game bird ration formulated to Wildlife International, Ltd.'s specifications. The feed was provided ad libitum during acclimation and during the test, except of an overnight starvation period of at least 18 hours prior to testing.
Acclimation:	Birds were given water soluble antibiotics in their drinking water for 6 days during the acclimation period. Approx. three weeks prior to test initiation.
<u>Test parameter:</u>	
Observations:	Mortalities, signs of toxicity, and behaviour were observed daily. Individual bodyweights were measured at the initiation of the test and on Days 3, 7, and 14 of the test. Average feed consumption was determined by pen for each dosage group and the control group for Days 0-3, 4-7, and 8-14. At test termination, all test birds were subjected to a gross necropsy.
Statistics:	There were only two mortalities in this study. Therefore, it was not possible to perform the calculation of a LD_{50} value. The LD_{50} value was determined to be greater than the highest dosage tested. No statistical analysis was applied to separate mean responses among treatment groups for feed consumption. Bodyweight data were compared by Dunnett's t-test using TOXSTAT software.
<u>Findings:</u>	
Mortalities:	No mortalities occurred in the control group or in the 295, 486, 810 or 1350 mg/kg treatment groups. However, there was 20% (2 out of 10) mortality in the 2250 mg/kg bw treatment group. One female in the control group was noted as ruffled in appearance approximately 2 hours after dosing possibly as a result of handling stress. Within 30 minutes the hen appeared normal and remained so throughout the test period. All other control birds were normal in appearance and behaviour throughout the test.
Clinical signs:	Signs of toxicity were observed at all levels, but resolved by day 10 in all groups. These signs included ruffled appearance, depression and lethargy.
Body weight, feed consumption:	Some slight reduction in food consumption was observed at doses above 295 mg/kg bw. Treatment-related reductions in body weight were observed in both

males and females.

At necropsy, pale liver and spleen and autolysis throughout the abdominal cavity were found in the two birds that died at the highest dose.

Table 9.1-7: Mortality and growth (bodyweights and food consumption) of northern bobwhite following acute oral exposure (gavage)

Test substance [mg/kg]	Cumulative mortality	Sex	Mean bodyweight change [g/bird]				Mean food consumption [g/bird/d]		
			0 to 3	3 to 7	7 to 14	total	0 to 3	4 to 7	8 to 14
Control	0	♂	+7	0	+2	+9	29	25	19
	0	♀	+12	-4	+2	+10	38	31	22
295	0	♂	+5	0	+2	+7	22	27	20
	0	♀	+4	+5	+1	+9	26	24	19
486	0	♂	-4	-4	+8	-1	17	21	18
	0	♀	5	+6	+1	+11	19	26	19
810	0	♂	-5	+4	+9	+9	12	16	15
	0	♀	-7*	-4	+18	+6	15	20	22
1350	0	♂	-10*	-3	+16	+3	15	21	19
	0	♀	-14*	+6	+12	+4	19	21	21
2250	2	♂	-21*	-1	+21	-3	13	29	28
	0	♀	-17*	0	+18	+1*	16	18	22

Conclusion: LD_{50} > 2250 mg ai/kg bw, NOEL could not be determined , no mortality dose was 1350 mg/kg bw

Comment RMS:

Validity: The study was evaluated according to the current valid test guidelines OCSPP 850.2100 (2012) and OECD 223 (2010).

Check of validity criteria:

OCSPP 850.2100 (2012):

- Birds were randomly assigned to treatment and control pens. Fulfilled.
- Not more than 10% of the control birds died during the test. The control in the current study was 0%. Fulfilled.
- At least ten birds were used for each dose level of the test substance and control. Fulfilled.
– The test substance was orally administered, via either capsule or gavage. Fulfilled.
- A minimum of five dose levels of the test substance, plus an appropriate control were tested. Fulfilled.

OECD 223 (2010):

	- The mortality in the controls should not exceed 10 per cent. In the current study the mortality was 0%. **In addition, the following points deviated from the test guideline or were not reported in detail:** - According to OCSSP 850.2100 the photoperiod should be 10 hours light and 14 hours dark. In the current study the photoperiod was 8 hours light and 16 hours dark as recommended in OECD 223. - According to OECD 223 the birds should weigh at least 180 g. In the current study the weight of the birds ranged between 169 and 213 g. **Acceptability of the analytical methods used in the test:** No analytical methods were reported. **Endpoints:** The RMS agrees on the endpoints given in the study report. LD_{50} is > 2250 mg/kg bw No LD_{10} or LD_{20} values could be determined due to the lack of mortality. **Conclusion of the RMS:** Based on the evaluation of the study the bird acute toxicity test is considered valid.

B.9.1.1.2. Short-term dietary toxicity to birds

According to the data requirements for active substances (Commission Regulation (EU) 283/2013) and/or plant protection products (Commission Regulation (EU) 284/2013) no short-term dietary toxicity tests are required to address the risk to birds. No new short-term dietary toxicity studies were submitted for the re-newal of the active substance triticonazole. The results of the short-term dietary studies summarised in the DAR (2000) are given below.

[redacted] ***(1992a) RPA 400727: Subacute dietary Toxicity (LC50) to Bobwhite Quail, Document No. R013037***

Guideline: US EPA FIFRA 71-2, OECD 205

GLP: Yes

Material and Methods:

Groups of 10 quail chicks (Colinus virginianus) were offered diets containing 163, 325, 650, 1300, 2600 and 5200 mg triticonazole/kg diet (Batch DA 640, purity 95.9 %). Two similar sized control groups were offered basal diet alone. The stability, homogeneity and spot concentrations of the test diets were conformed by analysis. Test diets were introduced at a bird age of 10 days and were offered to the birds for 5 days, then, test diets were replaced with basal diet and the birds observed for a further 3 days.

Assessments were made of mortality, clinical signs, bodyweight change and food consumption. At the end of the observation period an autopsy was carried out on ten birds from the highest surviving dose group (5200 mg ai/kg diet) and from the control group.

Findings:

Mean measured concentrations in the test diet were within 3.5 % of nominal. A single mortality occurred in the 1300 mg/kg dose group on day 3. Autopsy revealed no abnormalities. No other mortalities occurred. There were no other clinical signs of toxicity. Bodyweight and food consumption were reduced in the 2600 and 5200 mg/kg groups during days 1 - 5. For the last 3 days of treatment body weight gain was similar to the controls.

The subacute dietary LC50 of triticonazole to bobwhite quail was determined to be in excess of 5200 mg/kg diet. According to recommendations given in SANCO/4145/2000 this figure was converted to 693 mg/kg bw/d. Based on the single mortality, the NOEC was determined to be 650 mg/kg diet.

[redacted] ***(1992b): RPA 400727: Subacute dietary Toxicity (LC50) to Mallard Duck, Document No. R013038***

Guideline: US EPA FIFRA 71-2; OECD 205

GLP: Yes

Material and Methods:

Groups of 10 9-day old mallard ducklings (Anas platyrhynchos) were offered diet containing 163, 325, 650, 1300, 2600 and 5200 mg triticonazole/kg diet (Batch DA 640, purity 95.9%). Two similar sized control groups were offered basal diet alone. Assessments were made of mortality, clinical signs, body weight increase and food consumption. At the end of the observation period an autopsy was carried out on ten birds from the highest dose

group (5200 mg ai/kg diet) and 5 birds from the control group.

Findings:

Mean measured concentrations in test diets were within 13% of nominal concentrations throughout the 5 day dosing period. There were no mortalities or other clinical signs of toxicity recorded and there was no effect on food consumption. Body weight gain during the treatment period was reduced relative to control values at 2600 and 5200 mg/kg diet during days 1 - 5 of the treatment period. For the last three days of treatment these two groups were similar to controls. No abnormalities were observed in any birds examined post-mortem.

The subacute dietary LC50 of triticonazole to mallard duck was determined to be in excess of 5200 mg/kg diet. The NOEC was found to be 1300 mg/kg diet based on body weight effects.

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

Two studies were provided for the Annex I inclusion process ([redacted] 1995 and [redacted], 1998). In the course of confirmatory data two further studies were submitted and reviewed, a 1-generation Reproduction Study on the Bobwhite (*Colinus Virginianus*) ([redacted], 2007) and a 1-generation Reproduction Study on the Bobwhite (*Colinus Virginianus*) with shortend exposure of four weeks ([redacted] 2008). One additional study was performed, which was not submitted during the first EU peer-review process [redacted], 2012a). The study was requested during the first EU peer-review process by the ECCO-Peer-Review Meeting. After re-evaluation of the previous studies, the RMS had some concerns regarding the validity of three of them. To address these concerns the applicant provided a position paper:

Reference:	**Triticonazole (BAS 595F) – Position Paper on the Preliminary Birds and mammals Risk Assessment by AGES for the EU AIR III Renewal of Triticonazole**
Author(s), year:	Ott K., Pascual J., Kragten S., Ristau K., 2016
Report/Doc. number:	BASF DocID 2016/1321104

The relevant parts of this position paper are presented in the respective passages of the dRAR and marked in *italic.*

Reference:	**BAS 595F (Triticonazole) – 1-Generation –Reproduction Study on the Bobwhite quail (*Colinus virginianus*) by Administration in the Diet**
Author(s), year:	[redacted] 2012a
Report/Doc. number:	BASF DocID 2011/1269059
Guideline(s):	OECD 206, US EPA § 71-4, U.S. EPA-OPPTS 850.2300
GLP:	Yes
Deviations:	According to study plan food mixes should be used for a maximum of 6 weeks. Erroneously the first food mix was used over a time period of 47 days. Additional samples of the first food mix for each test concentration were taken on the last day. Analytical results demonstrated that the test substance was stable in the food mix over the prolonged time period. Therefore this deviation had no effect on the study results. Please also refer to the commenting box below
Validity:	Acceptable

Material and methods:

Test substance	BASF 595 F (Triticonazole), Batch: COD-001440 Purity 91.3% (tolerance ± 1%), CAS no.: 131983-72-7
Test species:	Bobwhite quail (*Colinus virginianus*)
Number of organisms:	16 pens per concentration group with one male and one female per replicate (+ 2 spare replicates for replacements during the pre-egg-production period)
Weight, age:	Body weight between 184 – 190.4 g (males) and 189.4 – 193.3 g (females) at test initiation, 6 months
Type of test:	Reproductive toxicity
Applied concentrations:	Control (untreated diet), 50, 150, 450 mg ai/kg diet (nominal) corresponding to 3.63, 10.98 and 32.76 mg ai/bw/day, respectively
Analytics:	Concentration, homogeneity and stability of the test substance in the diet were sufficiently verified by analytical methods.
Type of application:	Test substance mixed in the diet, prepared in intervals of 5 or 6 weeks.
Phases of the study:	Acclimation (pre-treatment): week -2 to -1; Pre-egg laying phase: week 1 - 10; Egg-laying phase: week 11- 22
Time of exposure:	22 weeks

Test conditions:

Temperature / relative humidity:	Adult birds: 18.1-27.4 °C / 36-100% Egg storing: 15 - 18 °C / 51 – 83 % Incubation: acclimation to room temperature for about 1 day before incubation 37.5 ± 0.5 °C / 61 – 84 % Chicks: 37-43 °C in the center under the heaters, decreasing with increasing distance from the center / 36-100%

Lightning:	Week -2: 7 hours light, 17 hours darkness Week 1 to 7: 7 hours light, 17 hours darkness Week 8 to 9: 14 hours light, 10 hours darkness Week 10 to 22: 17 hours light, 7 hours darkness Illuminance: 20-80 lux
Feeding	All adult birds and their offspring were given feed and water ad libitum during acclimation and testing.
<u>Test parameter:</u>	
Observations:	The animals were monitored daily from arrival onwards. Signs of toxic effects and abnormal behaviour were monitored. One male bird died during the acclimation period. All other birds were of good health until day 0. One day before start of the scheduled egg-laying period (10 Aug 2011) the following cages were eliminated: Group 0: cages 7 (foot lesion) and 13 (comparably low body weight of one of the birds) Group 1: cages 26 and 35 (comparably low body weight of one of the birds) Group 2: cages 38 and 44 (comparably low body weight of one of the birds) Group 3: cages 55 (male died during acclimation period) and 59 (comparably low body weight of one of the birds)
Adult Body weight:	Adult body weights were determined at the start of the treatment period (day 0) and after 2, 4, 6, 8 weeks of the pre-egg-laying period, and at adult termination. Offspring body weights were determined at hatching and at 14 days of age after CO_2 asphyxia.
Adult feed consumption:	Starting with the treatment period the total food consumption per replicate was determined once weekly by measurement of the weight of the food containers at start and end of the week. The mean value per bird and day was calculated. Observations concerning palatability were made on a routinely basis. No impairment of feed uptake was observed. Therefore no detailed records were made.
Egg parameter:	Records were maintained for each pen and each week of the numbers of eggs that were laid, cracked, abnormal. Eggshell thickness was recorded for each pen and every two weeks. The number of infertile eggs per pair and week, embryonic death at day 11 and 18 by candling and chicks “dead-in-shell” at hatching were recorded
Hatchling parameter:	Records were kept of the numbers of hatchlings and offspring surviving for 14 days (14-day survivors) per pen, per week. Any abnormalities in chicks hatched were recorded.
Necropsy:	Adult birds which died during the treatment period and birds that were sacrificed at the end of the treatment period were necropsied and subjected to gross-

pathological assessment. Birds, which were sacrificed at the end of the pre-egg-laying period and those, which were terminated because the male or the female bird in a replicate had died were not examined.

Birds were sacrificed by CO_2 asphyxia.

For the chicks no post-mortem examination was carried out.

Statistics: Descriptive statistics; Dunnett-test for body weight and food consumption of parent quails, for the egg weight, egg shell thickness and chicks' body weight, and for comparing each dose group with the control group. Wilcoxon-test for count data and proportions ($\alpha = 0.05$, $\alpha = 0.01$).

Findings:

Analytical results: The achieved concentrations, stability and homogeneous distribution of the test substance in the diet for 40 days at room temperature were confirmed as acceptable, i.e. in the range of 89.1 % and 95.7 % of nominal concentration.

Biological effects: There were no mortalities of adult quails observed in any concentration group during the whole exposure period, nor over the whole pre-egg-laying and the egg-laying period. Generally, the birds were in good health throughout the experimental period except isolated findings, mostly moderate lesions from fighting and consequential injuries (e.g. foot and eye lesions were noted during the macroscopic pathological examination). Clinical signs attributable to the test substance were not observed.

In comparison to the control, the food consumption was statistically significantly increased in test group 1 in week 5 and in test group 2 in weeks 1 and 2. In test group 3 a statistically significant decrease was observed in week 19. All these deviations were considered to be incidental. Over the whole exposure period no trend towards an increase or decrease of the food consumption was observed in any of the tested concentration groups.

The body weights of the exposed birds were not markedly increased or decreased in comparison to the control group. The statistical analyses revealed no evidence of any treatment-related effect for all measurements and both sexes.

The egg shell thickness of the treatment groups was at about the same level as in the control group. No statistically significant deviation from the control group was identified.

Percentage of damaged eggs: Following revised animal welfare regulations, this test was carried out in markedly larger test cages with a floor of Noryl® instead of wired mesh. In these cages the eggs were available for the parent birds and the structure of the floor facilitated picking of eggs, since the parent birds could easily hold the eggs on one place. Thus the rate of damaged eggs was very high up to week 6 of the egg-laying period. From week 7 on the floor was completely covered with card board, on which the eggs could roll, and the percentage of

damaged eggs was markedly reduced. However, the normal values for the proportion of cracked eggs of eggs laid provided by the test guideline (i.e. 0.6 – 2.0%), which were based on experience gained in smaller cages with egg catchers, could not be obtained in the large cages. Nevertheless the proportion of damaged eggs in the control group of this study (6.3%) is considered to be in an acceptable range and to have no influence on the overall sensitivity of this study. The proportion of damaged eggs of eggs laid in the highest concentration group was low (4.3%) and the parameter was not affected by the test substance in previous studies conducted with the same test substance.

Generally the fertility was comparably low in the first part of the egg-laying period. No statistically significant deviation to the control group was identified in any of the treatment groups.

The total embryonic mortality was calculated as sum of early and late embryonic mortalities. The statistical evaluation was performed by analysis of the proportion of viable eggs set on day 18 of fertile eggs. The evaluation did not indicate any treatment-related effect on embryonic survival.

At 450 mg active ingredient/kg diet (daily dose 32.76 mg active ingredient/kg bodyweight and day) following statistically significant effects were observed:

- Reduced number of eggs laid per female quail and week
- Reduced proportion of 14-day-old surviving chicks of chicks hatched

In consequence of these reductions:

- Reduced number of hatched chicks per female quail and week
- Reduced number of 14-day-old-surviving chicks per female quail and week
- Reduced proportion of 14-day surviving chicks of eggs initially set and of fertile eggs

The body weight at hatch is a parameter which was affected by the test substance in earlier studies and is thus of specific interest. However, in this study a statistically significant decrease in comparison to the control group was only observed in group 3 (450 mg ai kg diet) for weeks 5-8, but not over the whole egg-laying period. However, a slight trend towards a lower body weight in the highest concentration group was visible.

At 150 mg active ingredient/kg diet (daily dose 10.98 mg active ingredient/kg bodyweight and day) following statistically significant effects were observed:

- The proportion of chicks dead-in-shell of fertile eggs which was statistically significantly increased in comparison to the control group in weeks 9 – 12 and for the whole egg-laying period (Wilcoxon-test). The difference was statistically significant only on the 5% level and no effect was seen at the higher treatment

level.

- The proportion of hatched chicks of eggs set at day 18, which reflects the proportion of chicks dead-in-shell, was statistically significantly decreased in weeks 9 – 12, but not over the whole egg-laying period (Wilcoxon-test). An analysis of the individual data shows that there was no clear trend over the whole egg-laying period and that the high proportion of chicks that died in the last week of the egg-laying period (28 of 66 viable 18-day embryos = 42.4%) contributed markedly to the deviation. For both parameters the highest concentration group (the 450 mg ai/kg diet) was clearly not affected. No concentration-related trend could be seen. In conclusion, the statistically significant increased proportion of chicks dead-in-shell can be regarded as incidental and may have been caused by specific unfavourable conditions for the eggs in that test group during the last hatch.
- Survival of chicks after hatch was slightly lower than in the control group, but not statistically significantly reduced over the whole egg-laying period. Only during the second part of the egg-laying period the reduction was statistically significant. It was concluded, that the survival after hatch was affected by the test substance in the highest concentration group only since in the 150 mg ai/kg diet group the effect was not significant over the whole egg-laying period.

Effect over the whole embryonic development in 150 mg ai/kg diet group was considered to not be treatment-related.

In summary, the most sensitive endpoints were the egg production and the survival after hatch as shown in the 450 mg ai/kg diet group. Statistically significant differences from the control group in 150 mg ai/kg diet were driven by the incidentally increased proportion of chicks “dead-in-shell” and were not considered to be treatment related.

Statistical power: As the test was conducted according to the current guidelines, the statistical power is considered to be acceptable.

Table 9.1-8: Summary of effects of triticonazole on the reproduction of the bobwhite quail (Colinus virginianus)

	Experimental group [mg ai/kg diet]			
	Control	**50**	**150**	**450**
Number of replicates	16	16	16	16
No. treatment-related mortality of adult birds	0	0	0	0
Adult body weight [g] (male/female) end of egg laying period	216.1/226.1	219.1/234.7	211.3/238.8	213.5/225.2
Body weight change [%] over total period (day 0-154)	17.45/19.38	15.74/23.33	10.98/23.54	14.54/16.93

	Experimental group [mg ai/kg diet]			
	Control	**50**	**150**	**450**
No. of eggs laid / group	892	913	883	569
No. of cracked and broken eggs / group	63	56	79	22
Mean egg weight (g) [a]	9.9	10.1	10.1	9.8
Mean egg shell thickness (mm) [a]	0.20	0.19	0.20	0.19
No. of eggs set [b]/ group	750	771	723	476
No. of fertile eggs / group	706	736	662	455
No. of infertile eggs / group	44	35	61	21
No. of early embryonic mortalities / group	28	27	24	14
No. of viable 11-day old embryos / group	678	709	638	441
No. of late embryonic mortalities / group	13	14	8	12
No. of viable 18-day old embryos / group	665	695	630	429
No. of total embryonic deaths / group	41	41	32	26
No. of "dead-in-shell" / group	146	116	152	86
No. of chicks hatched / group	519	579	478	343
No. of 14-day surviving chicks / group	424	466	356	206
No. of eggs laid / female bird / week [a]	4.6	4.8	4.6	3.0**
No. of eggs laid per hen/day	0.66	0.68	0.66	0.43**
No. of chicks hatched / female bird / week [a]	2.7	3.0	2.5	1.8**
No. of 14-day surviving chicks / female bird / week [a]	2.2	2.4	1.9	1.1**
Mean body weight of chicks at hatching (g) [a]	6.2	6.3	6.1	6.0
Mean body weight of chicks 14 days after hatching (g) [a]	23.7	23.9	23.3	22.4
% fertile eggs of eggs set [b]	92.8	95.3	92.6	93.5
% viable 11 day old embryos of eggs set [b]	89.4	91.7	89.2	90.5
% viable eggs at day 18 of eggs set [b] at day 11	98.3	98.2	98.7	97.4
% hatched chicks of eggs set at day 18	78.9	83.3	74.3	78.9
% 14-day survivors of chicks hatched	79.4	79.6	73.1	55.3**
% cracked and broken eggs of eggs laid	6.3	6.0	8.5	4.1
% early embryonic mortalities of fertile eggs	3.6	3.9	3.7	3.2
% late embryonic mortalities of fertile eggs	1.6	1.7	1.2	2.4
% "dead-in-shell" of fertile eggs	19.7	15.6	24.3*[c]	19.5
Hatchability (% chicks hatched of total eggs set)	69.3	75.1	65.8	70.1
Hatchability (% chicks hatched of fertile eggs)	75.1	78.9	70.8	74.9

[a] mean values calculated as means from mean values of each replicate
[b] incubated
[c] this deviation was considered to be not test substance related
* statistically significant differences compared to the control ($p \leq 0.05$)
** statistically significant differences compared to the control ($p \leq 0.01$)

Conclusion: In a bobwhite quail avian reproduction test with triticonazole the NOEL was determined at 10.98 mg ai/kg b.w./day (NOEC = 150 mg ai/kg).

Comment RMS:	**Validity:** The study was evaluated following the recommendations of the currently valid test guideline OECD 206 (1984) and EPA - OCSPP 850.2300 (2012). **Check of validity criteria:** OCSPP 850.2300 (2012): - Birds were randomly assigned to treatment and control pens. Fulfilled. - Not more than 10% of the control birds died or became moribund during the test. The control mortality during the test was 0%. Fulfilled. - The average number of eggs laid per hen in the control group was at least 29 for northern bobwhite or mallard. The eggs laid per hen in the control were 55 Fullfilled. - The number of viable embryos in the control group was at least 80% of the eggs set for northern bobwhite. In the current test it was 89.4%. Fulfilled. - The number of 18 day old northern bobwhite embryos of eggs set in the control group was at least 97%. In the current test it was 98.3%. Fulfilled. - The number of normal hatchlings in the control group was at least 85% of the viable embryos for northern bobwhite. In the current study it was 78.9%. - The number of normal hatchlings in the control group was at least 71% of the eggs set for northern bobwhite. In the current study it was 69.3%. - The number of 14 day old survivors in the control group was at least 77% of the normal hatchlings for northern bobwhite. In the current study it was79.4%. Fulfilled. - The average eggshell thickness in the control group is at least 0.20 mm for northern bobwhite. In the test the average eggshell thickness was 0.20 mm. Fulfilled. - The percentage of cracked eggs in the control group is not more than 13%. In the control group 7% of the eggs were cracked. Fulfilled. OECD 206 (1984): - The mortality in the controls should not exceed 10 per cent at the end of the test. During the study mortality 0%. Fulfilled. - The average number of 14-day old survivors per hen in the controls should be at least 12 for bob white quail. In the current study it was 22. Fulfilled. - The average egg shell thickness for the control group should be at elast 0.19 mm for bobwhite quail. In the current study it was 0.2. mm. Fulfilled. **In addition, the following points deviated from the test guideline or were not reported in detail:** - According to OECD 206 the proportion of cracked eggs of eggs laid in the control should not exceed 0.6 – 2.0 %. In the current study the proportion of cracked eggs

was 6.3%. An argumentation by the test facility explaining the high percent of cracked eggs is given in the study report above. However, as the validity criteria are met the study is assumed to be acceptable.

- According to OECD 206 the adult birds should be maintained at 22 ± 5°C and 50 to 75% relative humidity. In the current study temperatures fluctuated between 18.1 and 27.4 °C and were for 3 days and 15 minutes in total above and for 8 days and 20 hours and 15 minutes below the set limits of 21±2°C. According to the study report, temperatures above 24°C were only observed for short time periods during the day. However it is not specified how a short time period is defined. The situation was similar with the humidity, fluctuating between 36 and 100 % with total time above the limit of 41 d and 11 hours and a total time below the limit for 17d, 17 h and 30 minutes. According to the study report the humidity was only occasionally slightly below the limit values of 45% and above the limit of 70% due to the daily cleaning of the room. Again it is not defined what occasionally or slightly means in this regard.

One day before the start of the scheduled egg-laying period some replicates were replaced with the spare replicates because one of the birds showed comparably low body weight. It is questionable if this replicates should have remained in the test as the weight loss theoretically could be an effect of the treatment. However as the weight losses were occasional and distributed over the control and all of the treatments it may be assumed that they were not treatment related.

Acceptability of the analytical methods used in the test: Evaluation of the analytical methods (water/sediment/soil) according to the Guidance Document SANCO/3029/99 rev. 4.

Method: HPLC

Linearity: Calibration is based on duplicate determination at 5 concentrations, calibration range 47-440 ppm; r^2 not reported

Accuracy: 7 fortification level, 2 measurements; RSD: 7.3%

Precision: 92.5%

LOQ: 1.06 mg/ (lowest concdentration in calibration), corresponding to 10.6 mg/kg feed

LOD: not reported

The analytical method fulfils the requirements of guideline SANCO/3029/99 rev. 4.

Endpoints:

Slight effects were observed at 150 mg/kg diet, however these effects are not considered treatment related. Therefore the RMS agrees on the endpoints given in the study report.

NOEL = 10.98 mg ai/kg bw/day

LOEL = 32.76 mg ai/kg bw/day (450 mg ai/kg diet)

	The reporting of an EC_x is neither required nor mentioned by guideline OECD 206 or OCSPP 850.2300. EFSA (2013)[1] rates both guidelines with 3, which actually means that the NOEL should be maintained as standard endpoint. EC_x- values were therefore not calculated. An assessment of the statistical power was not performed and is not considered necessary as the test design is in line with the recommendation of OECD 206. **Conclusion of the RMS:** According to the OCSPP 850.2300 the study would not be valid regarding the number of normal hatchlings in the control group (78.9% of the viable embryos instead of 85%; and 69.3% of the eggs set instead of 71%) However, these deviations are considered acceptable in the light of the fact that according to OECD 206 all validity criteria are met. Based on the evaluation of the study the bird reproduction test is considered valid. The Co-RMS expressed concerns regarding the reliability of the study due to the fact that it did not meet all the validity criteria of OCSPP 850.2300 (2012). Furthermore the Co-RMS stressed that there is a potential dose/concentrations and treatment related effect on the number of 14-day surviving chicks/femals per week. Whilst the effect at 150 ppm isn't statistically significant it is fitting a dose/concentrations respond and could be potenially be treatment related. The RMS in principle aggrees with the Co-RMS however, the effect is small (13.6%), not statistically significant and all other parameters do not show effects. However it would be appreciated to get the opinons of the other member states regarding this issue during peer review.

Reference:	**The Reproductive Toxicity Test of RPA 400727 In Northern Bobwhite (*Colinus Virginianus*)**
Author(s), year:	[redacted]., 1995a
Report/Doc. number:	R013161
Guideline(s):	FIFRA Subdivision E, Section 71-4, OECD 206 (1984)
GLP:	Yes
Deviations:	Text reference Section 5.8 Husbandry and Environmental Conditions: During the test all birds will be maintained at an ambient temperature of approximately 21 ± 4°C Deviation: Chart recorders were used to record temperatures. On 4 occasions, the chart does not reflect a 360 degree circle. These incidences were due to technician error – Impact on study: none Text reference Section 5.8 Husbandry and Environmental Conditions: Photoperiod

[1] EFSA (European Food Safety Authority), 2015. Technical report on the outcome of the pesticides peer review meeting on general recurring issues in ecotoxicology. EFSA supporting publication 2015:EN-924. 62 pp.

	will be 7 hours of light per day during the first 16 weeks. The photoperiod will then be increased over a period of at least 7 days to 16 hours light per day to induce egg laying. Deviation Photoperiod was increased over a period of six days to 16 hours light and 8 hours dark. On 03/27/95, the photoperiod was increased to 17 hours of light and 7 hours of dark. Reason for deviation: The photoperiod was up to 16 hours of light by the 6th day. The photoperiod was extended to 17 hours of light to conform to laboratory procedure for reproductive studies. Please also refer to the commenting box below
Validity:	acceptable

Material and methods:

Test substance	Triticonazole technical (RPA 400727), Batch no.: 9316201, Purity 91.9%, CAS no.: 131983-72-7
Test species:	Northern Bobwhite (*Colinus virginianus*)
Number of organisms:	16 pairs with one male and one female per cage,
Weight, age:	140-250 g at test initiation, 17 weeks
Type of test:	Reproductive toxicity
Applied concentrations:	Control (untreated diet), 250, 500, 1000 ppm ai
Analytics:	Concentration, homogeneity and stability of the test substance in the diet were sufficiently verified by analytical methods.
Type of application:	Test substance mixed in the diet, prepared on an as needed basis
Phases of the study:	Acclimation (pre-treatment): 9 weeks; Pre-photostimulation: 7 weeks; Pre-egg laying (with photostimulation): 4 weeks; Egg-laying: 10 weeks; Post-adult termination (final incubation, hatching, and 14-day offspring rearing period): 5 weeks
Time of exposure:	21 weeks
Test conditions:	
Temperature / relative humidity:	Adult housing: 23.4 ± 1.23 °C / 54.2 ± 5.6% Egg storing: no information on temperature and humidity provided Incubation: no information on temperature and humidity provided Hatching: no information on temperature and humidity provided Offspring management: approx. 38.9 °C / no information on humidity provided
Lightning:	Weeks 1-15: 7 hours light:16 hours dark Week 16 onwards: photoperiod increased to 16 hours of light per day. After approximately 26 weeks the photoperiod was increased to 17 hours of light per day and was maintained at that length until the adult birds were euthanized. Illuminance: approx. noon-day sunlight – 4870° Kelvin
Feeding	All adult birds and their offspring were given feed and water ad libitum during

acclimation and testing. The basal diet contained at least 20% protein and 2.5% fat, and not more than 7% fibre.

Test parameter:

Observations: During the study, all adult birds were observed daily for signs of toxicity or abnormal behaviour.

Adult Body weight: Adult body weights were measured at beginning of acclimation, at test initiation, at 13 and at adult termination. Offspring body weights were determined at hatching and at 14 days of age for surviving ducklings.

Adult feed consumption: Feed consumption for each pen was measured for a 7-day period every week throughout the test. Attempts were made to minimize feed wastage.

Egg parameter: Eggs were collected daily from all cages, when available, and marked according to the cage of origin and date of collection. Records were maintained for each pen and each week of the numbers of eggs that were laid, cracked eggs, eggs set, fertility and viability (after approx. 18 days). Eggshell thickness was recorded for each pen and week as appropriate.

Hatchling parameter: Records were kept of the numbers of hatchlings and offspring surviving for 14 days (14-day survivors) per pen, per week.

Necropsy: Adult birds that died or were euthanized during the course of the study were subjected to a gross necropsy. At the end of the exposure period, all surviving adult birds were euthanized with carbon dioxide gas and necropsied.

Statistics: Upon completion of the study, the data was first tested for homogeneity of variance using Batlett's Test. Proportional data was examined following Arcsine transformation. An ANOVA analysis was performed on the data for various parameters. Tukey's post-hoc test was then performed only in response to a significant result from the ANOVA for the purpose of directly comparing each of the test treatments to the control. In the event the data for a given parameter did not pass the Bartlett's Test for homogeneity of variance, a nonparametric ANOVA (Kruskal-Wallis) was used to determine treatment differences.

Findings:

Analytical results: Samples collected during the test to measure the achieved test substance concentrations for the 250, 500, and 1000 diets had percent of nominal values in the range of 112.9% to 119.3% at the time of preparation.

Biological effects: Not treatment-related mortalities occurred in all control and treatment groups. During the 21 week period there were 13 incidental mortalities predominantly caused by obvious cage injury. No overt signs of toxicity or effects upon adult body weight were observed. There were no significant differences in feed consumption among treatment groups. There was a statistically significant difference between the control and the 1000 ppm test group in the following

reproductive parameters: number of hatchlings/ number of eggs set, number of hatchlings/ number of fertile eggs and 14 day hatchling weights. There was a statistically significant difference between the control and both the 1000 and 500 ppm test groups in the parameters number of eggs laid per day, number of eggs set in the incubator, number of 14 day survivors/ number of set eggs, number of 14 day survivors/ number of hatchlings and hatchling weights. Specifically, the 500 and 1000 ppm groups laid 31 % and 53 % fewer eggs per day than the control and produced 32 % and 80 % fewer 14-day surviving chickens. There were no treatment related effects on numbers of fertile eggs, numbers of cracked eggs or number of viable embryos.

The estimated test substance intakes for bobwhite quails during the test were 16.9, 16.9, 16.2 and 16.6 mg ai/kg bw/d for the control, 250, 500 and1000 pm treatment groups, respectively. No apparent treatment related necropsy findings were recorded.

Table 9.1-9: Mean group body weight from a bobwhite quail dietary reproduction study

Test concentration [ppm]	Sex	Start of acclimation	Start of test feed Week 0	Start of egg lay Week 11	Test end Week 26
Control	Male	170.3	208.9	223.6	219.2
	Female	171.6	203.3	216.9	239.3
250	Male	171.1	201.8	218.6	216
	Female	175.7	209.1	221	236.4
500	Male	172	208.6	222.8	200.7
	Female	167	197.5	218.4	227.8
1000	Male	178.4	214	226.7	220.2
	Female	176.8	207.1	225.1	232.2

* Significantly different from the control at $p < 0.05$

Table 9.1-10: Summary of reproductive results in the bobwhite quail

Reproductive parameter	Test concentration [mg ai/kg diet]			
	control	250	500	1000
Number of replicates	14	14	13	13
Total eggs laid	670	664	426	292
Eggs cracked	3	2	4	0
Eggs set	607	595	363	241
Fertile eggs	554	520	311	183
Viable embryos	543	508	297	170
Hatchlings	517	492	260	136
14-day old survivors	457	403	155	25

Reproductive parameter	Test concentration [mg ai/kg diet]			
	control	250	500	1000
Eggs laid/hen/week	4.79	4.74	3.28*	2.25**
Eggs laid/hen/day	0.64	0.63	0.44*	0.3*
Number of eggs set into incubator	0.58	0.57	0.37*	0.25*
Mean eggshell thickness measurements [mm]	0.216	0.224*	0.219*	0.223*
Mean hatchling bodyweight [g]	7.0	6.9	6.3*	5.8*
Mean 14-day old survivor bodyweight [g]	29.6	29.7	28.5	23.3*
Eggs cracked/eggs laid [%]	0.4	0.3	0.9	0
Viable embryos/fertile eggs [%]	98	97	95	92
Hatchlings/fertile eggs [%]	93	94	83	74*
14-day old survivors/hatchlings [%]	88	82	60*	18*
Hatchlings/eggs set [%]	85	82	71	56*
14-day old survivors/eggs set [%]	75	67	42*	10*

* Significantly different from the control at $p < 0.05$
** Significantly different from the control at $p < 0.01$

Conclusion: 250 ppm is considered as the NOAEC (a slight, not statistically significant tendency of lower reproductive performance is indicated in some parameters at 250 ppm, see table above). This corresponds to a NOAEL of 19.5 mg/kg bw.

Comment RMS:

Validity: The study was evaluated following the recommendations of the currently valid test guideline OECD 206 (1984).

Check of validity criteria:

- The mortality in the controls should not exceed 10 per cent at the end of the test. During the study mortality 9.4%. Fulfilled.
- The average number of 14-day old survivors per hen in the controls should be at least 12 for bob white quail. In the current study it was 28. Fulfilled.
- The aberatge egg shell thickness for the control group should be at elast 0.19 mm for bobwhite quail. In the current study it was 0.216. mm. Fulfilled.

In addition, the following points deviated from the test guideline or were not reported in detail:

- According to OECD 206 the age of the birds at the beginning of the test should be 20-24 weeks. In the current test the birds were 17 weeks old at beginning of the test. During the 21 week period there were 13 incidental mortalities predominantly caused by obvious cage injury. The cages in which mortality occurred were not used in any statistical comparisons.

However, 3 deaths in the control were caused by quail pox and it is questionable if the birds were in a healthy condition in general. Furthermore 4 of the deaths in the treatment groups showed signs of emaciation. One of these birds in the treatment group of 250 ppm even died of starvation. The cause of the emaciation is not fully

evident and it is not clearly stated if this birds were examined post-mortem.
The applicant provided following argumentation (Position Paper by Ott, Pascual Kragten & Ristau, 2016).

13 incidental mortalities

Concerning mortality, the number of dead birds in the control was within the valid range of 10% stated in the guidelines. Also the overall mortality rate (over all treatment groups) was around 10%. The causes of death were generally incidental with two cases of diagnosed quail pox, but mainly based on injuries due to pen-mate aggression. The latter is a well known issue in this study type (see draft revision of the OECD Guideline 206, April 2000). Since the fatalities were incidental, evenly distributed across all dose levels and not treatment related, it is considered justified to exclude them from the statistical analysis. The remaining birds (13-14 bird pairs per group) were sufficient to fulfill the OECD 206 guideline requirements of 12 pairs per treatment group.

General health condition of birds and cause of emaciation

The observed emaciation reported for four birds were in three cases considered secondary to severe injuries due to pen-mate aggression. The death of the single bird that probably starved to death is considered coincidental because the general health status of the birds was good and no effects on body weight or food consumption were observed in the birds of the same dose group.
Considering the health of the remaining birds, the reproductive parameters of the control were compared with the normal values stated in the OECD Guideline 206. Since these were well within the given ranges and even in the upper range, and since the body weight and egg-production was well in line with the other available triticonazole studies (see Appendix 1), it can be concluded that the health status of the overwhelming majority of the birds tested was not impaired.

Conclusion by the applicant

In conclusion, the re-evaluation of the report and particularly of the raw data allows for a better understanding of the results and for supporting the validity of the study. First, the observed increase in fatalities were not treatment related, still within the normal range, and fulfilled the validity criteria for the control of the OECD 206 and the more recently updated EPA guideline (<10%). Second, the data for the control were within the normal range for reproductive parameters given in OECD 206: egg production and number of hatchlings was even above the OECD 206 range while the % cracked eggs was below the normal range. And third, the observed emaciation in birds was mainly secondary due to severe injuries or incidental in one single case.
Taking into account the evaluation and acceptance of this study in the previous EU peer-review and the re-evaluation of study data and the explanations provided here

by BASF to address the concerns raised recently (2016) by the RMS, BASF considers that this study is valid according to current test guidelines (i.e., OECD 206). Consequently, the toxicity endpoint derived from this study, the EU agreed endpoint of NOEL= 19.5 mg a.i./kg bw/d, should be used in the reproductive risk assessment for birds in the ongoing AIR III evaluation of triticonazole.

Acceptability of the analytical methods used in the test: Evaluation of the analytical methods (water/sediment/soil) according to the Guidance Document SANCO/3029/99 rev. 4.

Method: Gas chromatorgraphy

Linearity: Calibration 5 - 5000 ppm; r^2 0.999

Accuracy: 3 fortification levels with each two measurements at two times; average recoveries 79.26% at 5 ppm and 103% at 5000 ppm.

Precision: Coefficient of variation 4.58% at 5 ppm and 2.68% at 5000 ppm

LOQ: 0.25µg/mL

LOD: 0.125µg/mL

The analytical method fulfils the requirements of guideline SANCO/3029/99 rev. 4.

Endpoints:

The RMS agrees on the endpoint given in the study report.

NOAEL = 19.5 mg ai/kg bw/day

The reporting of an EC_x is neither required nor mentioned by guideline OECD 206 or OCSPP 850.2300. EFSA (2013)[1] rates both guidelines with 3, which actually means that the NOEL should be maintained as standard endpoint. EC_x- values were therefore not calculated.

An assessment of the statistical power was not performed and is not considered necessary as the test design is in line with the recommendation of OECD 206.

Conclusion of the RMS: Based on the evaluation and the argumentation of the applicant the bird reproduction test is considered valid.

The Co-RMS proposes to set the NOEC < 250 ppm as there were statistically significant effects on the mean eggshell thickness at this test concentrations. However the egg-shells were thicker than in the control which is not considered to be an adverse effect at the values lie in the range as stated in the guideline OECD 206 for bobwhite quail (0.19-0.24 mm)

Furthermore the Co-RMS noted that whilst there are non-statistically significant effects on the number of 14-day old survivors/egg set, there is a potential dos/concentrations and treatment related effect that warrants further consideration.

The RMS in general agrees with the Co-RMS, however the effect is only marginal above 10% (10.6%) and all other parameters do not show effects > 10%.

However it would be appreciated to get the opinons of the other member states regarding this issue during peer review.

Reference:	**The Reproductive Toxicity Test of RPA 400727 with the Mallard Duck *(Anas platyrhynchos)***
Author(s), year:	[redacted] 1998b
Report/Doc. number:	R000098
Guideline(s):	EPA Guideline No.: 71-4 (b) (1982)
GLP:	Yes
Deviations:	Please refer to the commenting box below
Validity:	Acceptable

Material and methods:

Test substance	RPA-400727 (Triticonazole) Batch no.: OP9750057, Purity 90.52%, CAS no.: 131983-72-7
Test species:	Mallard duck (*Anas platyrhynchos*)
Number of organisms:	16 pens with one male and one female per group
Weight, age:	800- 1377 g at test initiation, 21 weeks and two days
Type of test:	Reproductive toxicity
Range finding:	Control, 100, 200, 400 and 800 ppm – no adult mortalities. Reproductive parameters for cracked eggs, eggs laid and eggs fertile were tested with no statistical differences noted.
Applied concentrations:	Control (untreated diet), 125, 250, 500 and 1000 ppm ai
Analytics:	Concentration, homogeneity and stability of the test substance in the diet were sufficiently verified by analytical methods.
Type of application:	Test substance mixed in the diet
Phases of the study:	Acclimation (pre-treatment): 2 weeks; Duration of Adult Exposure prior to egg laying: 11 weeks and 1 day (start of photostimulation after approx. 8 weeks)
Time of exposure:	21 weeks and one day
Test conditions:	
Temperature / relative humidity:	Adult housing: 20.1 ± 2.9 °C (17.2 – 29.4 °C) / 73 ± 10.4 % (37 – 90 %) Egg storing: 5.9 °C / no information on humidity provided Incubation: 37.5 ± 0.1 °C (37.3 – 37.6 °C) / 56.3% (53-59 %) Hatching: 37.1 ± 0.2 °C (36.9 – 37.6 °C/ 77.2 ± 0.8 % (75 – 79 %) Offspring management: 34.4 ± 3.6 °C /32.1 %
Lightning:	During acclimation and for the first 8 weeks of treated feed: 7 hours light:17 dark Week 9 over a 6-day period: gradually increase to 17 hours light maintained at that length until the end of the treated feed portion of the study. Illuminance: fluorescent lights, approx. to natural sunlight
Feeding	All adult birds and their offspring were given feed and water ad libitum during acclimation and testing. The basal diet contained at least 20% protein and 2.5%

crude fat, and no more than 7% crude fibre.

Test parameter:

Observations: During the study, all adult birds were observed daily for signs of toxicity or abnormal behaviour. Additionally, all offspring were observed for behaviour and mortality once daily from hatching until 14 days of age.

Adult Body weight: Adult body weights were measured at start of the acclimation , at start of treated feed, at start of photostimulation and at adult termination. The weight of the ducklings was determined at hatching and at 14 days of age for surviving ducklings after euthanization.

Adult feed consumption: Feed consumption for each pen was measured weekly throughout the treatment period for a total of 21 weeks and one day for a total of 22 feeding intervals.

Egg parameter: The numbers of eggs laid, cracked, number of fertile eggs on day 14 of incubation, number of viable embryos on day 21 of incubation. Eggshell thickness was recorded for each pen and week as appropriate.

Hatchling parameter: Records were kept of the numbers of ducklings hatched and were observed once daily for behaviour and mortality. Offspring surviving for 14 days (14-day survivors)..

Necropsy: Gross pathological examinations were performed on all birds succumbing prior to adult termination and on all birds surviving the. All surviving adult birds were euthanized by carbon dioxide asphyxiation.

Statistics: Upon completion of the study, statistical analyses were to determine statistically significant differences between the control group and each of the treatment groups. Sample units were the individual pens within each experimental group, except adult body weight where the sample unit was the individual bird. Data sets were first tested for normality using a Chi-square test and for homogeneity of variance using a Bartlett's test. Proportional data were Arcsine transformed. If the normality test and/or the homogeneity test did not pass, the data was tested with a Levene's Test for Homogeneity of Variance. Data sets were analysed with ANOVA and a post hoc test to test treatment means against control means with a Dunnett's Test (equal size of data set), a Tukey's Test (unequal size of data set) or with a Kruskal Wallis ANOVA (data set not normal and/or homogenous).

Findings:

Analytical results: Samples collected during the test to measure the achieved test substance concentrations for the 125, 250, 500 and 1000 ppm diets had percent of nominal values in the range of 119.99% to 94.37%, 111.14% and 92.83%.

Biological effects: Two mortalities occurred, one in the 125 ppm and one in the 250 ppm group, which were not related to treatment. No overt signs of treatment-related toxicity were observed in adult Mallard ducks during the exposition period. All birds were noted to be normal in appearance and behaviour. There were no observations made

on the post-mortem documentation regarding any toxicity-related effects.

No significant differences were detected for any of the adult or reproductive parameters tested.

Table 9.1-11: Mean group body weight from a mallard duck dietary reproduction study

Test concentration [ppm]	Sex	Start of acclimation	Start of test feed Week 0	Start of egg laying	Test end
Control	Male	1178.3	1142.9	1159.3	1196.8
	Female	985.8	989	1046.9	1161.1
125	Male	1203.0	1162.3	1185.3	1277
	Female	1020.3	978.8	1002.8	1226.5
250	Male	1133.3	1138.8	1170.9	1242.1
	Female	1003.7	970.8	1010.4	1204.3
500	Male	1173.8	1183.8	1192.3	1241.6
	Female	1002.3	985.9	1042.4	1214.8
1000	Male	1197.8	1151.6	1129.4	1197.1
	Female	1005.4	970.1	982.5	1114.6

* Significantly different from the control at $p < 0.05$

Table 9.1-12: Summary of reproductive results in the mallard duck

Reproductive parameter	Test concentration [mg ai/kg diet]				
	control	125	250	500	1000
Number of replicates	16	12	15	16	14
Total eggs laid	899	580	827	846	714
Eggs cracked	48	35	42	24	30
Eggs set	779	477	713	751	621
Fertile eggs	732	439	658	693	539
Viable embryos	678	368	580	614	489
Hatchlings	566	262	493	489	418
14-day old survivors	556	259	478	463	397
Eggs laid/hen/day	0.8	0.67	0.79	0.76	0.73
Number of eggs set into incubator/hen/day	0.7	0.57	0.68	0.67	0.63
Mean eggshell thickness measurements [mm]	0.368	0.376	0.360	0.362	0.361
Mean hatchling bodyweight [g]	36	35.9	36	37	36.7
Mean 14-day old survivor bodyweight [g]	149.7	239.1	169.5	156.5	185.8
Eggs cracked/eggs laid [%]	5	6	5	3	4
Viable embryos/fertile eggs [%]	93	84	88	89	91
Hatchlings/viable embryos [%]	83	71	85	80	85
14-day old survivors/hatchlings [%]	98	99	97	95	95
Hatchlings/eggs set [%]	73	55	69	65	67

Reproductive parameter	Test concentration [mg ai/kg diet]				
	control	125	250	500	1000
14-day old survivors/eggs set [%]	71	54	67	62	64

* Significantly different from the control at $p < 0.05$; cage pairs not laying any eggs are not included in this data presentation.

Conclusion: The reproductive NOEC during the study was 1000 ppm.

Comment RMS:

Validity: The study was evaluated following the recommendations of the currently valid test guideline OECD 206 (1984) and OCSPP 850.2300 (2012)

Check of validity criteria:

OCSPP 850.2300 (2012):

- Birds were randomly assigned to treatment and control pens. Fulfilled.
- Not more than 10% of the control birds died or became moribund during the test. The control mortality during the test was 0%. Fulfilled.
- The average number of eggs laid per hen in the control group was at least 29 for northern bobwhite or mallard. The eggs laid per hen in the control were 56 Fullfilled.
- The number of viable embryos in the control group was at least 80% of the eggs set for mallard duck. In the current test it was 87%. Fulfilled.
- The number of 21 day old mallard duck embryos of eggs set in the control group was at least 94%. In the current test this value is not stated.
- The number of normal hatchlings in the control group was at least 52% of the viable embryos for mallard duck. In the current study it was 83%. Fulfilled.
- The number of normal hatchlings in the control group was at least 44% of the eggs set for mallard duck. In the current study it was 73%. Fulfilled.
- The number of 14 day old survivors in the control group was at least 94% of the normal hatchlings for mallard duck. In the current study it was 98%. Fulfilled.
- The average eggshell thickness in the control group is at least 0.316 mm for mallard duck. In the test the average eggshell thickness was 0.36 mm. Fulfilled
- The percentage of cracked eggs in the control group is not more than 13%. In the control group 5.4% of the eggs were cracked. Fulfilled.

OECD 206 (1984):

- The mortality in the controls should not exceed 10 per cent at the end of the test. During the study mortality 0%. Fulfilled.
- The average number of 14-day old survivors per hen in the controls should be at least 24 for mallard duck. In the current study it was 34. Fulfilled.
- The average egg shell thickness for the control group should be at 0.34 mm for mallard duck. In the current study it was 0.36 mm. Fullfilled.

In addition, the following points deviated from the test guideline or were not

reported in detail:

- According to OECD 206 the humidity for the adult housing should be between 45 and 70%. In the current study it was 37-90%.

According to OECD 206 the temperature for hatchlings should be 22 – 35°C. In the current study it was 37.3-37.6°C.

Three pairs in the 125 ppm and two pairs in the 1000 ppm group did obviously not lay any eggs and were not included in the statistical evaluation. This fact was not mentioned in the "results" part of the study report and no explanation or comparison with historical data on the strain of ducks used was given. For these reasons an explanation from the Notifier was requested. In this statement [redacted] 2003;) it is argued that the validity criteria according to EPA and OECD concerning control productivity were met and no dose-response relationship in pairs laying no or few eggs could be identified. Furthermore it is stated that the occurrence of pairs laying no or few eggs is not uncommon in bird repro studies and not specific to triticonazole.

The RMS has the impression that the productivity of the 125 ppm group seems significantly lower compared to the control (21.6 14-day-old survivors per egg-laying hen, 3 pairs laying no eggs compared to 34.8 14-day-old survivors per hen in the control). But as no dose-response relationship is apparent there are no conclusive reasons to suppose a treament-related effect and it is considered as incidental. In an overall view the study is considered to be acceptable and valid.

Acceptability of the analytical methods used in the test: Evaluation of the analytical methods (water/sediment/soil) according to the Guidance Document SANCO/3029/99 rev. 4.

Method: Gas chromatography

Linearity: Calibration is based on four concentrations in a range oft 5 to 5.000 ppm

Accuracy: average recovery is 79.26% at 5 ppm and 103% at 5.000 ppm

Precision: coefficient of variation is 4.58% at 5 ppm and 2.68% at 5.000 ppm

LOQ: 0.2 µg/mL

LOD: 0.1 µg/mL

The analytical method fulfils the requirements of guideline SANCO/3029/99 rev. 4.

Endpoints:

The the RMS agrees on the endpoints given in the study report.

NOEC = 1000 ppm (corresponding to 108.15 mg/kg bw/day, based on the mean consumption of 117.98 g/day and an average body weight of 1090, 9 g).

The reporting of an EC_x is neither required nor mentioned by guideline OECD 206 or OCSPP 850.2300. EFSA (2013)[1] rates both guidelines with 3, which actually means that the NOEL should be maintained as standard endpoint. EC_x- values were therefore not calculated

	An assessment of the statistical power was not performed and is not considered necessary as the test design is in line with the recommendation of OECD 206. **Conclusion of the RMS:** Based on the evaluation of the bird reproduction test is considered valid.

Reference:	**BAS 595 F – 1-Generation Reproduction Study on the Bobwhite (*Colinus virginianus*) by Administration in the Diet**
Author(s), year:	[redacted] 2007
Report/Doc. number:	BASF DocID 2006/1026908
Guideline(s):	FIFRA Subdivision E, Section 71-4, U.S. EPA- OPPTS 850.2300, OECD 206 (1984)
GLP:	Yes
Deviations:	The birds were about 1 ½ month younger than recommended by the EPA guideline (7 month), but within the limits required by the OECD guideline (20-24 weeks). The fertility rate of the control group was within normal limits therefore the age of the test animals was considered to be appropriate for the study.
Validity:	Acceptable

Material and methods:

Test substance	BAS 595F Batch no.: COD-000601
Test species:	Bobwhite Quail (*Colinus virginianus*)
Number of organisms:	16 pairs with one male and one female per cage, 4 spare replicates
Weight, age:	About 199 to 2003 g, 5 ½ month
Type of test:	Reproductive toxicity
Applied concentrations:	Control (untreated diet); 170 (group1) and 300 (group 2) mg ai/kg feed (nominal). The concentrations were corrected for purity to reflect 100 % ai The daily dietary doses were 0, 12.4 and 21.7 mg ai/kg bw/day.
Analytics:	One sample from the upper, middle and lower layer was taken from the highest and the lowest concentration directly after the first mixing for the determination of the homogeneous distribution in the diet.
Type of application:	Test substance mixed in the diet, test substance mixtures were prepared in weekly intervals until 05 Jan 06, from then on the intervals between new mixes were prolonged to 5 weeks, since results from the stability analysis indicated a sufficient stability.
Phases of the study:	Acclimation: 2 weeks; Pre- Pre-egg laying: 10 weeks; Egg-laying: 12 weeks; Post-adult termination
Time of exposure:	22 weeks
Test conditions:	

Temperature / relative humidity:	Adult housing: 18.5 - 24 °C / 28-86% Chick housing: 40 ± 2°C Egg storing: 16 ± 1°C/ 60 – 90% Incubation: generally approx. 37.8 ± 0.1 °C/ generally approx. 59-69% Hatching: 37.7 - 37.9°C / 80 – 90 % Offspring management: 40 ± 2 °C / no information on humidity provided
Lightning:	Acclimation: Week -1: 7 hours light:17 hours dark Substance feeding period before egg-laying: Week 1 to 7: 7 hours light: 17 hours dark Week 8 to 9: 14 hours light: 10 hours dark Substance feeding 1 week before and during egg-laying period: Week 10 to 22: 17 hours light:7 hours dark Illuminance: 34-257 Lux
Feeding	All adult birds and their offspring were given feed and water ad libitum during acclimation and testing. The basal diet contained at least 24% protein and 6% fat, and not more than 4% fibre.
<u>Test parameter:</u>	
Observations:	During the study, all adult birds were observed daily for signs of toxicity or abnormal behaviour.
Adult Body weight:	Adult body weights were measured at beginning the treatment period (day 0), after 2, 4, 6 and 8 weeks of the pre-egg production period and at the end of the test. Offspring body weights were determined at hatching and at 14 days of age for surviving chicks.
Adult feed consumption:	The total food consumption/replicate was determined once weekly. The mean value/per bird and day was calculated.
Egg parameter:	Eggs were collected daily during the 12-week egg-laying period starting at the beginning of week 11 and ending at the end of week 22. The eggs were labelled with the cage number and the week collected. For each pen and each week the numbers of eggs that were laid and cracked eggs were recorded. The eggs, cracked ones included, were weighed per replicate and the weight and the number of eggs weighed was recorded. The eggs were candled to check for shell abnormalities, cracks ore breakages. Eggshell thickness was recorded for each pen and week as appropriate. All eggs placed in the incubator were candled approximately on days 11 and 18 of the incubation period for evaluation of infertilities, and early and late embryonic deaths.
Hatchling parameter:	Records were kept of the numbers of hatchlings and offspring surviving for 14 days (14-day survivors) per pen, per week.
Necropsy:	Adult birds that died or were euthanized during the course of the study were

necropsied and subjected to gross-pathological assessment. Birds, which were sacrificed at the end of the pre-egg laying period and those, which were terminated because the male or the female bird in a replicate had died were not examined. At the end of the exposure period, all surviving adult birds were euthanized with carbon dioxide gas and necropsied.

Statistics: The SAS system was used for data analysis (USEPA, 2002). A pairwise comparison of each dose group with the control was done via the Wilcoxon-test. The Dunnett`s test was applied for several parameters.

Findings:

Analytical results: Samples collected during the test to measure the achieved test substance concentrations for the 170 and 300 mg/kg diets had percent of nominal values in the range of 91.6% and 109% at the time of preparation.

Biological effects: Generally, the birds were in good health throughout the experimental period except isolated findings, mostly foot lesions. Clinical signs attributable to the test substance were not observed. Two females died in the weeks 11 to 22 (egg-laying period) treatment the group 2. One of the birds showed a head injury and macroscopic pathological findings were skinlesion on the head, liver rupture, coagulated blood in the abdominal cavity, the other one showed no clinical and no macroscopic pathological findings. The birds sacrificed at the end of the test did not show any treatment-related macroscopic pathological findings. No toxic signs caused by the test substance were seen in the chicks. A small part of the chicks (less than 2%) hatched had crippled feet or legs or a malformed spinal cord. The incidence was the same in all test groups including the control group and was clearly not treatment-related increased. No overt signs of toxicity or effects upon adult body weight were observed. The statistical analyses revealed a significant increase of food consumption in comparison to the control group in group 2 in weeks 5 and 8 ($p \leq 0.01$) and a significant decrease in week 17 ($p \leq 0.05$). However, no dose-related trend was observed and in conclusion the food consumption was not affected by the treatment with the test substance.

In the whole egg-laying period the mean number of eggs laid per female quail per week in group 2 (300 mg/kg diet) was not statistically significantly reduced, but a tendency towards a decreased egg-laying rate in comparison to the control group could be observed.

No impairment of the mean egg weight by the test substance occurred.

With 2.8% of broken eggs in the control, this parameter exceeded the value given in the OECD guideline (0.6-2%). The differences between the treatment groups and the control were not statistically significant.

The egg shell thickness of the treatment groups was at about the same level as in the control group.

The fertility rate was treatment-related decreased by the test substance in group 2. However, the fertility rate of group 2 was still in the range which is acceptable for the control group according to the test guidelines (75-90% of eggs set). There was no effect of the test substance on the embryonic survival until day 11 of the incubation. No statistically significant impairment of late embryonic survival occurred.

The rates of "dead in shell" of fertile eggs were 23.9% for the control, 27.7% for group 1 and 19.1% for group 2. No dose-related trend could be observed.

For the number of hatched chicks per female quail and week a statistically significant decrease in comparison to the control group was identified in group 2 for the whole egg-laying period. The proportion of hatched chicks of eggs initially set was statistically significantly decreased in comparison to the control group in concentration group 1 and in group 2 in the weeks 1-4.

Table 9.1-13: Summary of effects of titiconazole on the reproduction of the bobwhite quail (*Colinus virginianus*)

	Experimental group [mg ai/kg diet]		
	Control	**170**	**300**
Number of replicates	16	16	16
No. treatment-related mortality of adult birds	0	0	0
Adult body weight [g] (male/female) end of egg laying period	220.1/251.4	213.1/243.1	207.3/240
Body weight change [%] over total period (day 0-154)	8.85/25.83	5.44/21.73	2.88/19.82
No. of eggs laid / group	898	884	687
No. of cracked and broken eggs / group	24	20	15
Mean egg weight (g) [a]	10	9.7	9.8
Mean egg shell thickness (mm) [a]	0.2	0.21	0.2
No. of eggs set [b] / group	798	784	601
No. of fertile eggs / group	760	690	444
No. of infertile eggs/group	38	94	157
No. of early embryonic mortalities / group	34	46	2
No. of viable 11-day old embryos / group	726	644	442
No. of late embryonic mortalities / group	14	16	7
No. of viable 18-day old embryos / group	712	628	435
No. of total embryonic deaths / group	48	62	9
No. of "dead-in-shell" / group	179	202	103
No. of chicks hatched / group	533	426	332
No. of 14-day surviving chicks / group	363	265	134
No. of eggs laid / female bird / week [a]	4.7	4.6	3.6
No. of chicks hatched / female bird / week [a]	2.8	2.2	1.7*
No. of 14-day surviving chicks / female bird / week [a]	1.9	1.4	0.7

	Experimental group [mg ai/kg diet]		
	Control	170	300
Mean body weight of chicks at hatching (g) [a]	5.8	5.5*	5.4*
Mean body weight of chicks 14 days after hatching (g) [a]	19.4	18.9	18.5
% fertile eggs of eggs set [b]	93.9	84.8	78.5*
% viable 11 day old embryos of eggs set [b]	89.4	79.3	78.2
% viable eggs at day 18 of eggs set [b] at day 11	98.0	95.0	98.5
% hatched chicks of eggs set at day 18	74.5	69.9	80.4
% 14-day survivors of chicks hatched	65.5	56.2	37.8**
% cracked and broken eggs of eggs laid	2.8	2.4	6.1
% early embryonic mortalities of fertile eggs	5.2	5.7	0.3
% late embryonic mortalities of fertile eggs	1.9	4.8	1.5
% "dead-in-shell" of fertile eggs	23.9	27.7	19.1
Hatchability (% chicks hatched of total eggs set)	65.6	52.8*	61.2
Hatchability (% chicks hatched of fertile eggs)	69.1	61.8*	79

[a] mean values calculated as means from mean values of each replicate
[b] incubated
[c] this deviation was considered to be not test substance related
* statistically significant differences compared to the control ($p \leq 0.05$)
** statistically significant differences compared to the control ($p \leq 0.01$)

Conclusion: The overall reproductive performance, as expressed by the number of 14-day surviving chicks per hen was decreased in the 300 mg/kg group and as well in the 170 mg/kg group. However, in the group receiving 170 mg/kg diet this was considered to be caused by an increased embryonic mortality, which was not treatment-related. Therefore the decreased reproductive performance in this group was probably not treatment-related. NOEC = 170 mg /kg diet corresponding to a NOEL of 12.4 mg/kg bw/d.

Comment RMS: This study was submitted as confirmatory data in 2009.

Validity: The study was evaluated following the recommendations of the currently valid test guideline OECD 206 (1984). and OCSPP 850.2300 (2012)

Check of validity criteria:

OCSPP 850.2300 (2012):

- Birds were randomly assigned to treatment and control pens. Fulfilled.
- Not more than 10% of the control birds died or became moribund during the test. The control mortality during the test was 0%. Fulfilled.
- The average number of eggs laid per hen in the control group was at least 29 for northern bobwhite or mallard. The eggs laid per hen in the control were 56 Fullfilled.
- The number of viable embryos in the control group was at least 80% of the eggs set. In the current test it was 90.9%. Fulfilled.

- The number of 21 day old bobwhite embryos of eggs set in the control group was at least 94%. In the current test this value is not stated.
- The number of normal hatchlings in the control group was at least 85% of the viable embryos for bobwhite. In the current study it was 73.4%.
- The number of normal hatchlings in the control group was at least 71% of the eggs set for bobwhite. In the current study it was 65.6%.
- The number of 14 day old survivors in the control group was at least 77% of the normal hatchlings for bobwhite. In the current study it was 68%.
- The average eggshell thickness in the control group is at least 0.2 mm for bobwhite. In the test the average eggshell thickness was 0.2 mm. Fulfilled
- The percentage of cracked eggs in the control group is not more than 13%. In the control group 2.8% of the eggs were cracked. Fulfilled.

OECD 206:

- The mortality in the controls should not exceed 10 per cent at the end of the test. During the study mortality 0%. Fulfilled.
- The average number of 14-day old survivors per hen in the controls should be at least 12 for bob white quail. In the current study it was 33. Fulfilled.
- The average egg shell thickness for the control group should be at elast 0.19 mm for bobwhite quail. In the current study it was 0.2. mm. Fulfilled.

In addition, the following points deviated from the test guideline or were not reported in detail:

According to OECD 206 the broken eggs in the control should not exceed 2%. In the current study 2.8% of eggs were broken in the control. The differences between the treatment groups and the control were not statistically significant.

The applicant provided the following argumentation (Position Paper by Ott, Pascual, Kragten & Ristau, 2016):

2.8% broken eggs in control group

AGES is correct when stating that the percentage of cracked eggs in the control in the [redacted] (2007) study is with 2.8% above the normal range as given in the OCED Guideline 206 from 1984. However, considering the available information regarding this parameter, the real and technically feasible normal range is much higher than 2 % in these kind of studies. That has been acknowledged by the content of the more recent avian reproduction guideline by the US EPA (OCSPP 850.2300, version of 2012), which is a test guideline also recommended in EFSA 2009/1438. Thus, the slight exceedance observed I this study according to OECD 206 (1984) does not justify the invalidation of the study. A detailed reasoning is given below.

The control birds in this study produced a total of 898 eggs, of which 24 eggs were cracked. This results in 2.8% cracked eggs/eggs laid, which is just outside the range of 0.6 - 2% listed as normal for bobwhite quail in OECD 206. At the time this study

was carried out, the OECD 206 and EPA OPPTS 850.2300 guidelines did not include the parameter of percentage of cracked eggs as a must-be-met validity criteria, but listed it as a "normal value".

OECD 206 does not include information on how many studies, batches of birds, laboratories, or years of data were used to conclude that a normal percentage of cracked eggs for bobwhite quail is 0.6 - 2%, making the representativeness of this value questionable. OPPTS 850.2300 from 1996 stated that these values represent only a "few testing facilities". Indeed, OECD 206 says literally (and OPPTS 850.2300, substantially the same) that "These values are typical, but not necessarily representative for all facilities. If control birds do not meet or closely approach these values, the test procedures and conditions should be investigated for potential problems.". This point was clearly considered in the draft revision of OECD 206 of April 2000 which updated typical values for reproductive parameters, including 0 - 6% cracked/broken eggs for bobwhite quail (p. 15). Although the OECD 206 draft of April 2000 has not been adopted, it does recognises that the range of 0 - 6% is more realistic than the range provided in the original guideline (0 - 2%).

More conclusive is the content of the current version of the EPA Guideline (OCSPP 850.2300, version of 2012) which includes cracked eggs/eggs laid as a specific criterion for study validity. It states that a study is invalid if "there are greater than 13% cracked eggs in the control group." The 2.8% cracked eggs in [redacted] *(2007) study is well below the 13% cracked eggs required for a valid study under OCSPP 850.2300. Please note that OCSPP 850.2300 is a guideline officially accepted in the EU under the 1107/2009 legislation. In conclusion, the percentage of 2.8% of broken eggs in the control is not a reason to invalidate the study.*

According to OECD test guideline 206 at least three concentrations of the test substance have to be tested. The current study with only two concentrations tested does not fulfil this requirement which reduces the possibility to analyse the experiment using the proper statistical method. Based on statistical uncertainty the RMS questions the validity of the study.

The applicant provided the following argumentation (Position Paper by Ott, Pascual, Kragten & Ristau, 2016):

<u>Number of concentrations tested</u>

AGES raises the issue that only two instead of three treatment levels were tested and that this reduces the possibility for proper statistics. In the opinion of the notifier a test design with two test concentrations still allows proper statistics and the notifier does not consider it a reason to invalidate the study.

It is true that OECD Guideline 206 requires three dose levels, but it is not a validity criterion. The same is true for EPA Guideline OPPTS 850-2300. The main objective of these two guidelines is to derive a sound no effect concentration or level (NOEC

or NOEL). Statistically, two dose levels are sufficient to determine a NOEC/NOEL. OECD 206 and OPPTS 850.2300 ask specifically for a pairwise comparison of the treatment groups to the control group ('test groups should be individually compared to the control group', OCED 206, page 9). Thus, three treatment levels are not necessary to conduct a proper statistic and identify sound NOEC/NOEL values.

In this study, the statistical analysis were carried out using Dunnet's test for continuous data and Wilcoxon test for non-continuous data. Both tests compare pairs of data. Furthermore, these tests are applicable to two or three groups and therefore suitable to detect significant deviations in two-paired comparisons (i.e., between each treatment level and the control) and identify a NOEC/NOEL.

From a biologically perspective, having more than two treatment groups tested and use a wide range of treatment levels allow for a better understanding of the concentrations/doses causing conclusive effects and evaluate dose-response effects. This is one important reason for having more than two levels tested in regulatory ecotoxicological studies in general. But please notice that the desirable gain in power of a study by increasing the number of treatment levels tested needs to be balanced out with other factors (i.e., in the case of vertebrate studies, like birds, the number of animals tested, which is heavily influenced by the number of concentrations tested). For avian reproduction studies the number of treatment levels recommended is three. For triticonazole, in fact, such information (several concentration tested allowing for deriving a conclusive effect level) is available when the whole data package of avian reproduction studies is evaluated.

At the time when this study was carried out (2005/6), two further standard bird reproduction toxicity studies, one with mallard duck (BASF DocID R000098) and one with bobwhite quail (BASF DocID R013161) were available which were both considered valid in the Draft Assessment Report of 2005. Thus, historically, the current study was not considered as a stand-alone study, but meant to be seen in context with and supportive of the other already available studies. This is particularly important for the results of the bobwhite quail studies, the most sensitive species of the two tested. Higher concentrations, leading to conclusive effects, had been tested in the previous quail study (BASF DocID R013161). Beyond the situation at the time the study was carried out, the adequate approach now is to consider all the information currently available. […]

<u>Conclusion by BASF</u>

The slight increase in percentage of broken eggs and the fact that only two dose levels were tested do not appear to be reasons to change the conclusion of the previous EU peer-review of triticonazole which considered [redacted] 2007 a valid study (Addendum to the Confirmatory Data, 2009, p. 6-8). The study was carried out according to bird test guidelines which are still applicable under current legislation

with some modifications (i.e., cracked-eggs validity criteria) which confirm the validity of the study. Therefore, the [redacted] 2007 study should be considered as valid and its results adequate to derive a suitable toxicity endpoint for the use in the reproductive risk assessment for birds.

Acceptability of the analytical methods used in the test: Evaluation of the analytical methods (water/sediment/soil) according to the Guidance Document SANCO/3029/99 rev. 4.

Method: HPLC with external calibration

Linearity: Calibration based on duplicate determination at five concentrations; the 5 – 5000 ppm.

Accuracy: One fortificaton level with three or for measurements each at 4 different time points; Recovery 79.26% and 103%, respectively

Precision: 2 fortification levels; 4.58% at 5 ppm and 2.68% at 5000 ppm

LOQ: 1.06 mg/L, corresponding to 10.6 mg/kg feed

LOD: not reported

The analytical method fulfils the requirements of guideline SANCO/3029/99 rev. 4.

Endpoints:

The RMS agrees on the endpoints given in the study report.

NOAEL = 12.4 mg ai/kg bw/day

The reporting of an EC_x is neither required nor mentioned by guideline OECD 206 or OCSPP 850.2300. EFSA (2013)[1] rates both guidelines with 3, which actually means that the NOEL should be maintained as standard endpoint. EC_x- values were therefore not calculated.

Conclusion of the RMS: According to the OCSPP 850.2300 the study would not be valid regarding the number of normal hatchlings in the control group (73.4% of the viable embryos instead of 85%; 65.6% of the eggs set instead of 71%, and 77% of the 14-day old survivors instead of 68%).

However, these deviations are considered acceptable in the light of the fact that according to OECD 206 all validity criteria are met. Based on the evaluation of the bird reproduction test, the study is considered valid.

The Co-RMS noted that at 170 ppm the hatchability was significantly affected. Nevertheless, as the hatchability in the next higher concentration shows no statistically significant effect, and therefore no dose-response relation seems seems to be given.

However, the reliability of the study is questionable as only two concentrations were tested and it is not clear which consequences this has on the statistical power. Therefore, the RMS proposes to discuss the reliability of the study, its use in the risk assessment and the inclusion of its endpoint in the List of end points in the peer review or in an expert consultation.

Reference:	**BAS 595 F (Triticonazole),1-Generation Reproduction Study on the Bobwhite (*Colinus virginianus*) by Administration in the Diet for Four Weeks**
Author(s), year:	[redacted] 2008
Report/Doc. number:	BASF DocID 2008/1023059
Guideline(s):	Modified method based on OECD 206 taking into account the draft OECD test guideline "Avian Reproduction Toxicity test in Japanese Quail or Bobwhite Quail", April 2000
GLP:	Yes
Deviations:	None
Validity:	acceptable

Material and methods:

Test substance	BAS 595 F Batch no.: COD-000601
Test species:	Bobwhite Quail (*Colinus virginianus*)
Number of organisms:	20 pairs with one male and one female per replicate
Age:	6 ½ month
Type of test:	Reproductive toxicity
Applied concentrations:	Control (untreated diet; group 0); 300 (group 1) and 500 (group 2) mg ai/kg feed (nominal). The concentrations were corrected for purity to reflect 100 % ai The daily dietary doses were 0, 24.7 and 38.0 mg ai/kg bw/day.
Analytics:	One sample from the upper, middle and lower layer was taken from the high and the low concentration directly after the first mixing for the determination of the homogeneous distribution in the diet.
Type of application:	Each concentration was mixed separately. To prepare the supply for the whole 4 weeks exposure time, the test substance was mixed with the appropriate amount of feed in a laboratory mixer.
Phases of the study:	Acclimation: 3 weeks; Pre-egg laying: 2 weeks; Egg-laying: 8 weeks
Time of exposure:	4 weeks (2 weeks pre-egg laying and 2 weeks egg-laying period)
Test conditions:	
Temperature / relative humidity:	Adult housing: 20.2 - 23 °C / 20-69% Chick housing: 22 – 28.5°C/ 19-84% Egg storing: 16 ± 2°C/ 55 – 77% Incubation: generally 37.5 ± 0.2 °C/ generally approx. 62-77% Hatching: 37.3 - 37.7°C / 80 – 90 % Offspring management: 40 ± 2 °C / no information on humidity provided
Lightning:	Acclimation: Week -3: 7 hours light:17 hours dark; Week -2 to -1: 14 hours

	light:10 hours dark Substance feeding period before egg-laying: Week 1: 14 hours light: 10 hours dark Week 2: 17 hours light: 7 hours dark Substance feeding during egg-laying period: Week 3 to 4: 17 hours light: 7 hours dark Egg laying period without substance feeding: Week 5 to 10: 17 hours light: 7 hours dark Illuminance: 3-21 Lux
Feeding	All adult birds and their offspring were given feed and water ad libitum during acclimation (without test substance) and testing. The basal diet contained at least 23.7% crude protein and 6.9% crude fat, and not more than 3.2% fibre.
Test parameter:	
Observations:	During the study, all adult birds were observed daily for signs of toxicity or abnormal behaviour.
Adult Body weight:	Adult body weights were measured at beginning the treatment period, at the last day of the 2nd week (start of the egg-laying period) and at adult termination. Offspring body weights were determined at hatching and at 14 days of age for surviving chicks.
Adult feed consumption:	The total food consumption/replicate was determined once weekly. The mean value/per bird and day was calculated.
Egg parameter:	Eggs were collected daily during the 8-week egg-laying period starting at the beginning of week 3 and ending at the end of week 10. The eggs were labelled with the cage number and the week collected. For each pen and each week the numbers of eggs that were laid and cracked eggs were recorded. The eggs, cracked ones included, were weighed per replicate and the weight and the number of eggs weighed was recorded. The eggs were candled to check for shell abnormalities, cracks ore breakages. Eggshell thickness was recorded for each pen and week as appropriate. All eggs placed in the incubator were candled approximately on days 11 and 18 of the incubation period for evaluation of infertilities, and early and late embryonic deaths.
Hatchling parameter:	After about 21 days in the incubator, eggs were removed and placed in a hatcher. Records were kept of the numbers of hatchlings and offspring surviving for 14 days (14-day survivors) per pen, per week.
Necropsy:	Adult birds that died or were euthanized during the course of the study were necropsied and subjected to gross-pathological assessment. Birds, which were sacrificed at the end of the pre-egg laying period and those, which were terminated because the male or the female bird in a replicate had died were not examined.

Statistics: The SAS system was used for data analysis (USEPA, 2002). For the analysis of the body weight and of the food consumption of parent quails the DATATOX F1-System was used. For the body weight and food consumption of parent quails, for the egg weight, eggs shell thickness and chicks' body weight, a comparison of each dose group with the control group was carried out via two-sided Dunnett's test.

For count data and proportions, a nonparametric analysis was carried out. A pairwise comparison of each dose group with the control was performed via the one-sided Wilcoxon-test.

Findings:

Analytical results: Samples collected during the test to measure (first diet mix) the achieved test substance concentrations for the 300 and 500 mg/kg diets had a mean concentration of nominal values 91% and 110%.

Biological effects: Generally, the birds were in good health throughout the experimental period except isolated findings, mostly moderate lesions from fighting and subsequent injuries. Clinical signs attributable to the test substance were not observed. Three females died in the weeks 3 to 10 (egg-laying period), one in each group. The bird The bird in the group one was sacrified in extremies because of a foot lesion. The other two were found dead in the cage. Macroscopic pathological findings were Massive foot lesion on right side, moderate foot lesion on left side and head injury for the bird in group 0; massive both-sided foot lesion and cachexia for the bird in group 1 and a massive head injury and general bad condition for the bird in group 2.

The birds sacrificed at the end of the test did not show any treatment-related macroscopic pathological findings. No toxic signs caused by the test substance were seen in the chicks. A very low number of the chicks hatched had crippled feet or legs or a malformed spinal cord. The incidence was clearly not treatment-related increased. No overt signs of toxicity or effects upon adult body weight were observed.

The statistical analyses revealed a significant decrease of food consumption (-11%) in comparison to the control group in group 2 in the 4 weeks of the exposure period indicating a slight avoidance of the test substance by the birds. However, this did not result in an effect on the body weight development.

The mean number of eggs laid per female quail per week revealed no evidence of any dose effect. No impairment of the mean egg weight by the test substance occurred.

The proportion of cracked and broken eggs of total eggs laid was low with 0.9% in group 0 and 2 and 0.4% in group 1 and the statistical analyses revealed no evidence of any treatment effect.

The egg shell thickness of the treatment groups was at about the same level as in the control group.

There was no effect of the test substance on the fertility rate.

A statistically significant increase on comparison to the control group of the rate of early embryonic mortality of fertile eggs was observed in group 1 and group 2. However, in the study report it is argued that the rate of early embryonic mortality in the control group of this study was low in comparison to the range of historical controls (mean = 2.7 ± 2.3% standard deviation, range = 0.5-7.9% for 13 reproduction studies with bobwhite quails conducted according to the test guideline OECD 206 in the last 10 years.) The values of both test substance groups in this study were well in the range of the historical controls. Furthermore, early embryonic mortality was not affected by the test substance in earlier studies performed according to the standard protocol for avian reproduction tests. Therefore the slight increase in early embryonic mortality in the two concentration groups was not considered to be a treatment-related effect.

No statistically significant impairment of late embryonic survival occurred. However for the proportion of eggs set at day 18 of fertile eggs the Wilcoxon-test revealed a statistically significant decrease I comparison to the control group in dose groups 1 and 2 ($p \leq 0.05$). The decreases were a consequence of the higher rates of early embryonic mortality.

The rates of “dead in shell” of fertile eggs were 17.8% for the control, 12.8% for group 1 and 15.1% for group 2. No dose-related trend could be observed.

For the number of hatched chicks per female quail and week, for the proportion of hatched chicks of eggs initially set and for the proportion of hatched chicks of fertile eggs the statistical analyses revealed no evidence of any dose effect.

The proportion of 14-day old survivors of chicks hatched was statistically significantly decreased in comparison to the control group in concentration group 2 for the whole egg-laying period (-6%) ($p \leq 0.05$). the survival post hatch was also decreased by the test substance in high concentration levels in standard reproduction studies with the test substance in bobwhite quail. Therefore it is likely that the slight decrease seen in this study was substance-related.

For the proportion of 14-day old survivors of fertile eggs a statistically significant decrease in comparison to the control group was observed in concentration group 2 ($p \leq 0.05$) for exposure weeks 1-2, but not for the whole egg-laying period. This reflects the increased mortality after hatch, which is included in this proportion.

In conclusion, the survival of chicks after hatch was lightly affected by the test substance in the test group receiving 500 mg active ingredient/kg diet. However, the decrease was low and did not result in a statistically significant decrease of the number of 14-day survivors by hen.

The body weight of the chicks at hatch was slightly reduced during the egg-laying weeks in the exposure period and the following two weeks (-11 and -8%), but not over the total egg-laying period. This is one of the parameters that were affected in standard reproduction studies with the test substance. As for the survival after hatch a decrease was mainly observed during the exposure period. Therefore it was considered to be a treatment-related effect even if the effect was not statistically significant over the total egg-laying period.

For the body weight at day 14 the statistical analysis revealed no significant decrease in comparison to the control group over the whole egg-laying period.

In conclusion, the test substance caused a slightly decreased body weight at hatch in the 500 mg/kg group during the exposure period, which did not result in a statistically significant difference over the whole egg-lying period.

Table 9.1-14: Summary of effects of titiconazole on the reproduction of the bobwhite quail (*Colinus virginianus*)

	Experimental group [mg ai/kg diet]		
	Control	**300**	**500**
Number of replicates	20	20	20
No. treatment-related mortality of adult birds	0	0	0
Adult body weight [g] (male/female) end of egg laying period	205.8/229.2	203.8/230.7	198.8/219.2
Body weight change [%] over total period (day 0-154)	6.08/13.47	6.93/13.53	3.87/9.11
No. of eggs laid / group	762	803	761
No. of cracked and broken eggs / group	6	1	8
Mean egg weight (g) [a]	9.9	9.9	9.6
Mean egg shell thickness (mm) [a]	0.18	0.19	0.19
No. of eggs set [b]/ group	686	728	684
No. of fertile eggs / group	628	693	660
No. of infertile eggs/group	58	35	24
No. of early embryonic mortalities / group	4	12	14
No. of viable 11-day old embryos / group	624	681	646
No. of late embryonic mortalities / group	4	4	2
No. of viable 18-day old embryos / group	620	677	644
No. of total embryonic deaths / group	8	16	16
No. of "dead-in-shell" / group	97	88	105
No. of chicks hatched / group	523	589	539
No. of 14-day surviving chicks / group	497	542	468
No. of eggs laid / female bird / week [a]	4.8	5.0	4.9
No. of chicks hatched / female bird / week [a]	3.3	3.7	3.4
No. of 14-day surviving chicks / female bird / week [a]	3.2	3.4	3.0
Mean body weight of chicks at hatching (g) [a]	6.4	6.2	6.0

	Experimental group [mg ai/kg diet]		
	Control	**300**	**500**
Mean body weight of chicks 14 days after hatching (g) [a]	25.8	24.7	24.5
% fertile eggs of eggs set [b]	91	95.4	92.4
% viable 11 day old embryos of eggs set [b]	90.5	93.9	88.3
% viable eggs at day 18 of eggs set [b] at day 11	99.4	84.3	84.6
% hatched chicks of eggs set at day 18	84.4	87.0	83.7
% 14-day survivors of chicks hatched	92.2	91.0	86.8*
% cracked and broken eggs of eggs laid	0.9	0.4	0.9
% early embryonic mortalities of fertile eggs	0.6	1.7**	4.3*
% late embryonic mortalities of fertile eggs	0.7	0.6	1.0
% "dead-in-shell" of fertile eggs	17.8	12.8	15.1
Hatchability (% chicks hatched of total eggs set)	76.2	80.9	78.8
Hatchability (% chicks hatched of fertile eggs)	80.9	84.9	79.5

[a] mean values calculated as means from mean values of each replicate
[b] incubated
[c] this deviation was considered to be not test substance related
* statistically significant differences compared to the control ($p \leq 0.05$)
** statistically significant differences compared to the control ($p \leq 0.01$)

Conclusion:

No substance-related effects were observed in the test group receiving 300 mg/kg diet.

In the concentration group receiving 500 mg/kg diet, the proportion of 14-day survivors of chicks hatched was lightly but statistically significantly reduced (-6%). Also the body weight of hatched chicks was slightly reduced during egg-laying weeks 1-2 and for the egg-laying weeks 3-4 after exposure was terminated (-11 and -8%), but not over the total egg-lying period. The number of 14-day surviving chicks per hen, however, was not statistically significantly reduced.

In conclusion, the NOEL was 300 mg/kg diet corresponding to 24.7 mg/kg body weight.

Comment RMS:

This study was submitted as confirmatory data in 2009.

The bird reproduction study was conducted according to a modification of the OECD test guideline 206 (1984).

Check of validity criteria:

- The mortality in the controls should not exceed 10 per cent at the end of the test. During the study mortality 0%. Fulfilled.
- The average number of 14-day old survivors per hen in the controls should be at least 12 for bob white quail. In the current study it was 25.6. Fulfilled.
- The average egg shell thickness for the control group should be at elast 0.19 mm for bobwhite quail. In the current study it was 0.18 mm.

In addition, the following points deviated from the test guideline or were not

reported in detail:

According to OECD 206 the humidity should be in a range of 45-70%. In the adult housing the humidity was below the limit for 21 days, 16 hours and 15 minutes. In the chick housing the humidity was below the limit for 41 days, 21 hours. However, no adverse effects were observed on any of the birds or on the reproductive performance of the control group.

According to OECD test guideline 206 at least three concentrations of the test substance have to be tested. The current study with only two concentrations tested does not fulfil this requirement which reduces the possibility to analyse the experiment using the proper statistical method.

The applicant provided the following argumentation (Position Paper by Ott, Pascual, Kragten & Ristau, 2016):

The reason for the invalidation by AGES of the study by [redacted] 2008 (2008/1023059) is the same as given for the invalidation of the [redacted] 2007 study (2006/1026908), see above). Since this is a general criticism (use of two treatment concentrations rather than three), the statistical argument described by the notifier for [redacted] 2007 above applies also to this study ([redacted] 2008). Beyond this generic statistical reasoning the notifier provides here additional explanations of the particularities of the [redacted] 2008 study.

[redacted] (2008) is not a standard guideline study. It was rather considered a higher tier study addressing the more realistic exposure scenario via treated seeds. Thus, this modified reproduction toxicity study followed generally OECD 206, but as written in the report "the study design was adapted to the appropriate realistic exposure duration reflecting seed treatment uses in spring" (more details in MCP 10.1, AIR III dossier).

Three experimental groups were tested (one control and two treatment levels) and the statistical evaluation of the results was carried out using pair-wise Dunnet's or Wilcoxon test for continuous and count/ratio data, respectively. These methods are considered adequate as described in the section above. The low dose level of 300 mg/kg diet was identified as NOEC based on the significant decreased survival rate of hatched chicks in the high dose level of 500 mg/kg diet. A significant effect on the early embryonic death in the low dose group was not considered to be treatment related due to the relatively low rate of early embryonic deaths in the control group compared to the historical control data. Furthermore, the values of the treatment groups were well within the historical range (based on studies conducted according to OECD 206 in the previous 10 years in the same lab). Since this study did not follow the standard protocol of the other studies available with quails with triticonazole, it is not appropriate to merge the results of the treatment levels of this study with those of the standard tests. However, the results of this study were conclusive and therefore the study itself should be considered as

valid.

Acceptability of the analytical methods used in the test: Evaluation of the analytical methods (water/sediment/soil) according to the Guidance Document SANCO/3029/99 rev. 4.

Method: HPLC with external calibration

Linearity: Calibration based on duplicate determination at 3 concentrations; 2 mg/100 ml – 8 mg/100 ml

Accuracy: One fortificaton level with three or four measurements each at 4 different time points; Recovery was in a range of 90.8 – 109.6%

Precision: not reported

LOQ: 12.5 mg/L, corresponding to 125 ppm in feed

LOD: not reported

The analytical method fulfils the requirements of guideline SANCO/3029/99 rev. 4.

Endpoints:

The applicant proposes an endpoint of 24.7 mg ai/kg bw (300 mg ai/kg diet). However, there was a statistically higher embryonic mortality compared to the control in the 300 mg ai/kg diet treatment group. The information in the study report regarding historical controls was considered not sufficient for an argumentation by the RMS. The applicant provided more detailed data:

Historical control data – species: Bobwhite quail (Colinus virginianus):

study	*year*	*supplier*	*Proportion of early embryonic deaths of fertile eggs [%]*
71W0545/015133	*2004/2005*	*Küberich*	*2.4*
71W0611/045050	*2005*	*Küberich*	*6.1*
71W0622/055033	*2005/2006*	*Küberich*	*5.2*
71W0414/015148	*2006*	*Küberich*	*2.3*
71W0443/035078	*2006/2007*	*Küberich*	*2.4*
71W0189/045094	*2007/2008*	*Küberich*	*1.9*
71W0683/055080	*2008*	*Küberich*	*0.9*
71W0622/05W001	*2011*	*Küberich*	*3.6*

Total no of studies: 8	***Mean***	***3.1***
Number of pens of each study: 16	***Standard deviation***	***1.8***
	Median	***2.4***
Parameters were	***Minimum***	***0.9***

	presented as mean values
	Maximum ***6.1***
Comment RMS:	In-life date current study: 01.03. 2008 - 15.04. 2008 Control: 0.6% 300 mg/kg bw/d: 1.7%* 500 mg/kg bw/d: 4.3%* *significantly different to control The mean value of HCD for early embryonic death in studies conducted between 2004 and 2011 is 3.1% (±1.8) with a range from 0.9 to 6.1 %. The low control mortality of the study under evaluation (0.6%) is comparable to the control mortality observed in the second study from 2008 (0.9%). This supports the conclusion that the supplied test animals at that time (2007 - 2008) were in good health condition. In the study under evaluation a dose response has been observed from control to 500 mg/kg bw/d group. In both treated groups (300 and 500 mg/kg bw/d) the mortality was statistically significantly increased. While the mortality (1.7%) at 300 mg/kg bw/d was well below the mean of HCD (3.1%) this was not any more the case for the 500 mg/kg bw/d group. Based on the fact that 1) only two instead of three groups have been tested, 2) trend in dose-response has been observed, 3) the early embryonic mortality at 500 mg/kg bw/d exceeded the mean HCD and 4) tested animals in time period of 2007 to 2008 seemed to be in good health condition (obvious as low mortality in control data), a potential treatment-related effect at 500 mg/kg bw/d (mortality of 4.3%) cannot be excluded. The Co-RMS is of the opinion that a NOEL cannot be determined in this study considering it difficult to verify the argumentation of the applicant due to the data variability between the experimental studies. The RMS agrees with the comment of the Co-RMS. Further opinions are welcome. **Conclusion of the RMS:** Studies with shortened exposure duration are not common in the environmental risk assessment. It is questionable whether all reproductive phases have been assessed and if so whether there were sufficient to detect any effects. Therefore, the RMS proposes to discuss the general usability of this study for the risk assessment in an expert consultation. Furthermore only two concentrations were tested and therefore its reliability is questionable. If the study as such is considered reliable and usable for the risk assessment, the choice of the endpoint should also be discussed based on the early embryonic mortality.

B.9.1.2. Effects on terrestrial vertebrates other than birds

For detailed information please see Volume 3 part B 6 of this RAR

B.9.1.2.1. Acute oral toxicity to mammals

No additional studies were submitted for the re-newal of the active substance. A summary of the toxicity of triticonazole to mammals is given in Table 9.1-15.

Table 9.1-15: Acute toxicity of tritconazole to mammals

Test species	Test design	Ecotoxicological endpoints	Reference
Rat	Acute, oral	LD_{50} > 2000 mg ai/kg bw	[redacted] 1990

The endpoint from the acute oral study with rats for the active substance triticonazole is > 2000 mg ai/kg bw. No mortalities occurred at this dose and this endpoint is used for risk assessment.

Table 9.1-16: Acute toxicity of RPA 406341 to mammals

Test design	Test species	Ecotoxicological endpoint	Effects the Endpoint is based on	Reference
Oral acute	Rat	LD_{50} > 2000 mg/kg bw	decreased activity, reduced defacation	[redacted] 1999

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

No additional studies were submitted for the renewal of the active substance. A summary of the toxicity of triticonazole to mammals is given in Table 9.1-17.

Table 9.1-17: Long-term toxicity of triticonazole to mammals

Test design	Test species	Ecotoxicological endpoint	Effects the Endpoint is based on	Reference
Oral toxicity 28 days	Rat	$NOAEL_{males}$ = 1500 ppm corresponding to 152.3 mg/kg bw/d	↓Body weight gain	[redacted] 1991
		$NOAEL_{females}$ = 500 ppm corresponding to 52.4 mg ai/kg bw/d	↓absolute uterus weight	
Oral toxicity 90 days		NOAEL = 250 ppm corresponding to 19.8 mg ai/kg bw/d[2]	↓Body weight gain, ↓food consumption, ↑absolute and relative liver weight, ↑absolute and relative ovary weight,	[redacted] 1991

Test design	Test species	Ecotoxicological endpoint	Effects the Endpoint is based on	Reference
			necropsy findings in adrenals	
2-generation reproduction		NOAEL = 750 ppm corresponding to 48.41 mg ai/ kg bw	Parental: maternal mortality, ↓body weight, necropsy findings in adrenals, liver and ovaries ↓mating and fertility index in F1 generation Offspring: effects on survival and growth consistently observed across both generations	[redacted], 1993
Developmental toxicity		NOAEL = 200 mg ai/kg bw/d	↓maternal body weight gain, Foetal: ↑incidence of additional 13th and 14th rib	[redacted], 1991
	Rabbit	**NOAEL = 25 mg ai/kg bw/d[1]**	Paternal: maternal mortality, abortions, ↓food consumption, ↓body weight gain Foetal: Increased incidences of different skeletal findings	[redacted], 1991

Bold values were used for the risk assessment

[1] The toxicological endpoint is considered to be 5 mg ai/kg bw as at ≥ 25 mg ai/kg bw a slight body weight loss at days 6 to 8 and reduced food intake occurred. Furthermore one precocious ossification of acromiom process was noted at ≥ 25 mg ai/kg/d. However, the body weight loss and the reduced food intake were < 10% and not statistically significant and the precocious ossification is not considered ecotoxicologically relevant (please refer to Volume 3B-9 PPP).

[2] The dose spacing of this study was unfavourable as the distance between the dose for the NOAEL and the next higher dose was very high. (next higher dose: 50 mg/kg bw) Therefore, the NOAEL of 25 mg ai/kg bw is considered relevant for the ecotoxicological risk assessment.

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

Substances with a high potential to bioaccumulate in the food chain could bear a risk of secondary poisoning for birds and mammals if feeding contaminated prey like fish or earthworms. For organic chemicals, a log P_{OW} > 3 is used to trigger an evaluation of the potential for bioaccumulation.

The log P_{OW} value of the active substance triticonazole is 3.3 (Chabassol et al, 1991). The log P_{OW} for the

metabolite RPA 404766 is 1.6 (Cowlyn, 2014b), for RPA 406341 it is 2.2 (Cowlyn, 2014d), for RPA 407922 it is 1.9 (Cowlyn, 2014 c), for RPA 406203 it is 3.5 (Cowlyn, 2014e). Therefore for triticonazole and RPA 406203 the potential for bioaccumulation has to be assessed. For further details please refer to Volume 3 B9-CP.

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

There is no data requirement for reptiles and amphibians according to Regulation 283/2013 and 284/2013 under Regulation 1107/2009. Furthermore, there is currently no clear guidance for a risk assessment on reptiles and amphibians.

Considering the available information on birds and mammals and aquatic organisms an acceptable risk for reptiles and amphibians is indicated. In addition, there are also no indications of adverse effects of triticonazole on terrestrial vertebrate wildlife such as reptiles and amphibians in the literature review. Please see B.9.11.

B.9.1.5. Potential for endocrine disruption

Wild mammals

All endocrine-related mechanistic studies conducted by the notifier (*in vivo* and *in vitro*) or identified in the open literature are included in Section B6. The summary of all identified endocrine-related mechanistic level 2 and level 3/level 4 studies (according to the OECD Conceptual Framework for testing and assessment of endocrine discrupters; OECD TG 150) studies is followed by an assessment of treatment-related adverse effects with a potential endocrine mode of action.

Birds

The population relevant effects of triticonazole on birds were studied in reproductive toxicity studies on bobwhite quail and mallard ducks. However, endocrine effects on birds were not covered in the available toxicity tests. For a statement of the applicant regarding potential for endocrine disruption in birds please refer to Volume 3 B9-CP.

Amphibians and Reptiles

Currently no test methods are established to assess the population relevant effects of chemicals to amphibians or reptiles. While an amphibian metamorphosis test exists, this test was developed to evaluate the potential effect on the thyroid system, and not to measure population relevant effects. Therefore no further studies can be suggested at this time for these groups of organisms.

Under consideration of the lack of information it is not possible to draw a final conclusion on the endocrine disrupting potential of triticonazole. However, triticonazole is used as a seed treatment and therefore no contact exposure in terrestrial ecosystems for reptiles and amphibians is expected to occur.

B.9.2. EFFECT ON AQUATIC ORGANISMS

In order to complete the aquatic risk assessment and to address the new data requirements for active substances

(Regulation 283/2013) and plant protection products (Regulation 284/2013) according to Regulation (EC) no. 1107/2009, additional studies were performed. Further, tests on marine species, which were no data requirement according to the Regulation 91/414/EEC and hence were not evaluated during the first EU peer-review of the active substance were evaluated and summarised as well.

Studies submitted during the first EU peer-review of the active substance were evaluated according to the current valid test guidelines and are also summarised below.

B.9.2.1. Acute toxicity to fish

Reference:	**The acute toxicity of RPA 400727 to rainbow trout (*Oncorhynchus mykiss*)**
Author(s), year:	[redacted] 1990a
Report/Doc. number:	R013008
Guideline(s):	OECD test guideline 203
GLP:	Yes
Deviations:	Please refer to the commenting box below
Validity:	Acceptable

Material and methods:	
Test substance:	RPA 400727, batch no.: YG2156/1, purity: 99.5%
Test species:	Rainbow trout (*Oncorhynchus mykiss*)
Holding of fish :	No information available; acclimation to test conditions occurred but duration not known
Number of organisms:	2 replicates with 10 fish per replicate
Age, length, weight:	Age: no information, mean standard length: 4.4 cm (SD = 0.5), mean weight: 1.18g
Loading	0.59 g body weight/L fish loading per test vessel
Type of test:	Semi-static with daily medium renewal, 96 hours
Applied concentrations:	
Nominal:	0 (control and solvent control), 10 mg ai/L Because of the limited solubility of triticonazole the highest test concentrations did not exceed 10 mg ai/l.
Measured (mean):	- (control and solvent control), measured concentrations were between 9.190 and 9.93 mg/L for replicate 1 and between 9.345 and 9.529 mg/L for replicate 2, respectively.
Solvent:	Tween 80-dimethylformamide (100 µL/L)
Test conditions:	
Water quality:	Laboratory tap water, dechlorinated by the addition of sodium thiosulphate, total hardness: 350 mg /L as $CaCO_3$

Temperature:	14 ± 1°C
pH:	8.0-8.4
O_2 content:	10-10.1 mg/L
Light regime:	Light/dark cycle of 16/8, light intensity: no information available
Feeding	Commercial trout pellets, daily. The fish were not fed during the 96 hours study period.
Methods:	The test was carried out in glass aquaria holding 20 litres of test media
Test parameters:	All test vessels were monitored for mortality effects after 3, 6, 24, 48, 72 and 96 hours. Measurements of temperature, pH, and dissolved oxygen were made in all treatment solutions at the start and end of the test and after 24, 48, and 72 hours. In addition, temperature was continuously measured in the control vessel.
Analytical measurements:	The concentration of RPA 400727 in test solutions was determined on three occasions at 0, 24 and 96 hours
Statistics:	As no mortality occurred, no LC_{50} could be derived.
Findings:	
Analytical data:	The mean RPA 400727 concentration over the study period was between 92 and 99% of the nominal test concentration. Hence, the results of the study are based on nominal test concentrations.
Conclusion:	Neither in the control nor in the solvent control mortality was observed. No mortality was observed at this concentration, thus the calculation of LC_{50} was not possible and results in LC_{50} > 10 mg/L.

Comment RMS:	The study was evaluated following the recommendations of the currently valid test guideline OECD 203 (1992). **Check of validity criteria:** - The mortality in the control(s) should not exceed 10 per cent (or one fish if less than ten are used) at the end of the test. During the study mortality occurred neither in the control nor in the solvent control. Fulfilled. **In addition, the following points deviated from the test guideline or were not reported in detail:** - The total hardness in the test is 350 mg/L as $CaCO_3$ and therefore exceeding the recommended range of 10 to 250 mg/L in the guideline. - Because of the limited solubility of triticonazole the highest test concentrations did not exceed 10 mg ai/l. **Acceptability of the analytical methods used in the test:** Evaluation of the analytical methods (water/sediment/soil) according to the Guidance Document SANCO/3029/99 rev. 4. In the study very limited information is given regarding analytical methods

	Method: HPLC Linearity: 1.11-13.87 mg/L RPA 400-727 dissolved in mobile phase r^2 not reported Accuracy: One fortificaton at 9.977 mg/L, recovery ≥ 90% Precision: 7% LOQ: - LOD: - The analytical method does not fulfil the requirements of guideline SANCO/3029/99 rev. 4 but is acceptable. **Endpoints:** The RMS agrees on the endpoints given in the study report. LC_{50} > 10 mg/L nominal concentrations NOEC = 10 mg/L nominal concentrations **Conclusion of the RMS:** Based on the evaluation of the study the acute fish toxicity test is considered valid.

Reference:	**Triticonazole acute toxicity (96 hours) to rainbow trout (*Oncorhynchus mykiss*) under flow-through conditions**
Author(s), year:	[redacted] 1998a
Report/Doc. number:	C017670
Guideline(s):	E.P.A./FIFRA Guideline 72-1 (1985)
GLP:	Yes
Deviations:	Please refer to the commenting box below
Validity:	Acceptable

Material and methods:

Test substance:	Triticonazole lot no: 013951, purity: 972 g/kg
Test species:	Rainbow trout (*Oncorhynchus mykiss*)
Holding of fish :	Dilution water as used in the test; acclimation period of 14 days in 450 l glass tanks providing at least one liter of dilution water per fish. The mortality of the batch from which these test fish were taken was less than 5% during the 7 days prior to test initiation and less than 3% in the 48 hour period prior to test initiation.
Number of organisms:	2 replicates of 10 fish per concentration and control
Age, length, weight:	Representative sample (n=10) - Age: no information, mean total length: 4.15 cm (3.9-4.4), mean weight: 0.66 g (0.57-0.77)
Loading	0.09 g biomass/L of flowing test solution per 24 hours. fish
Type of test:	Flow-trough, 96 hours

Applied concentrations:

Preliminary range finding test:	0.12, 0.37, 1.11, 3.33 and 10.0 mg/L
Definitive test Nominal:	0 (control and solvent control), 0.48, 0.81, 1.4, 2.4 and 4 mg ai/L. The nominal concentration of 4.0 mg/l was above the visual limit of aqueous solubility. This was defined by the presence of undissolved particles in this test solution at test initiation.
Measured (mean):	- (control and solvent control), measured concentrations 0.48, 0.80, 1.4, 2.3, 3.6 mg/L
Solvent:	dimethylformamide
Test conditions:	
Water quality:	Tap water filtered through active charcoal and diluted with reverse osmosed deionized water), total hardness: 40±10 mg /L as $CaCO_3$ (48 mg/L at test initiation)
Conductivity:	133.5 µS/cm (at test initiation)
Temperature:	12.4-13.0°C
pH:	7.3-7.7
O_2 content:	9.4 mg/L
Light regime:	Light/dark cycle of 16/8, light intensity: no information available
Feeding	Commercial trout pellet twice daily by automatic distributors except during at least 24 hours prior to testing. The fish were not fed during the exposure period.
Methods:	14 inert plastic containers of 10 L each. Test concentrations were maintained by introducing between 5-10 aquarium volumes per day of newly prepared test solution via a constant flow system consisting of peristaltic pumps providing fixed flow rates of dilution water approx. 50 ml/min and syringe pumps providing fixed flow rates of test substance stock solutions (approx. 5µl/min).
Test parameters:	All test vessels were monitored for effects after 4, 24, 48, 72 and 96 hours. Measurements of specific temperature, pH, and dissolved oxygen were made in all treatment solutions at the start and once daily at each treatment level and in the controls throughout the exposure period.
Analytical measurements:	Samples of each test concentration and control were analysed at test initiation and 24 hours after test termination for determination of the test substance concentrations.
Statistics:	No mortalities occurred, therefore estimation of an LC_{50} was not possible.
Findings:	
Analytical data:	Measured concentrations were 88-108% of the nominal concentrations. However, in the study report the results were based on mean measured concentrations.
Test solution renewal system	The fixed flow rates of the stock solutions were 5 µL/min. The fixed flow rate of the dilution water was approximately 50 ml/min. During the test period the measured flow rates of test renewal system delivered 7.0 to 7.5 volume

	replacements per aquarium every 24 hours.
Biological findings:	No mortalities or significant sublethal toxic effects were observed in the dilution water control and solvent control groups during the test period. Significant sublethal effects were observed at the mean measured concentrations of 2.3 and 3.6 mg/l. These effects included erratic swimming.
Conclusion:	Based on mortality data, the LC_{50} was reported to be greater than the highest mean measured concentration of 3.6 mg/l after 96 hours of exposure. Based on biological observations, the NOEC for rainbow trout is reported to be 1.4 mg/L.

Comment RMS:	The study was evaluated following the recommendations of the currently valid test guidelines OPPTS 850.1075 (1996) and OECD 203 (1992). **Check of validity criteria:** OPPTS 850.1075 (1996): - Maximum-allowable control or solvent control mortality is 10 percent (or 1 mortality if 7 to 10 control fish are used) for a 96-h period of testing. If the test is continued past 96 h, the maximum-allowable additional mortality is 10 percent. During the study mortality occurred neither in the control nor in the solvent control. Fulfilled. - Constant conditions must be maintained throughout the test period. Flow-through procedures are preferred over static-renewal or semistatic procedures and static-renewal procedures are preferred over a static test procedure. The study was a flow-through test and constant conditons were maintained. Fulfilled. - In flow-through test, the dissolved oxygen (DO) should be maintained above 75 percent saturation. The O2-content in the study was 9.4 mg/L (90-92%). Fulfilled. - Measured concentrations are required if the test chemical is unstable or a flow-through diluter system is employed. In any case there must be evidence that test concentrations remained at least 80 percent of the nominal concentrations throughout the test or that mean measured concentrations are an accurate representation of exposure levels maintained throughout the test period. During the test, measured concentrations were 88-108% of the nominal concentrations. Fulfilled. OECD 203 (1992): - The mortality in the control(s) should not exceed 10 per cent (or one fish if less than ten are used) at the end of the test. During the study mortality occurred neither in the control nor in the solvent control. Fulfilled. **In addition, the following points deviated from the test guidelines or were not reported in detail:** - According to OPPTS 850.1075 (1996) the fish should not be fed for 48 hours

before test initiation. The report states that the fish were not fed for at least 24 hours before test initiation.

- According to OPPTS 850.1075 (1996) the temperature should be tested hourly in at least one replicate throughout the test. The measurements in all replicates were conducted at the start and every 24 hours throughout the exposure period.
- According to OPPTS 850.1075 (1996) the test substance should be measured in each replicate at 0, 48, and 96 h but chemical analysis was only conducted at test initiation and 24 hours after test termination. However the concentrations remained at 93 to 108% of the nominal concentrations.
- According to OECD 203 the temperature range should be 13-17 °C but the temperature was most of the time below 13 °C. However it was constant within in a range of 2 °C.

Acceptability of the analytical methods used in the test: Evaluation of the analytical methods (water/sediment/soil) according to the Guidance Document SANCO/3029/99 rev. 4.

Method: HPLC/UV

Linearity: Calibration is based on single determination at 5 concentrations

The calibration function was linear within the range from 20 to 1000 µg/L with r^2>0.999986

Accuracy: 5 fortification levels (each 3 measurements): 5 µg/L (1 x LOQ), 50 µg/L (10 x LOQ), 1 mg/L, 20 mg/L

Mean recoveries for each level: 103%, 100%, 102%, 105% and 99%

Precision: The relative standard deviation per fortification level is ≤ 20 %

LOQ: 5 µg/L

LOD: -

The analytical method fulfils the requirements of guideline SANCO/3029/99 rev. 4.

Endpoints:

The RMS agrees on the endpoints given in the study report.

LC_{50} > 3.6 mg/L mean measured concentration

NOEC = 1.4 mg/L mean measured concentration

Conclusion of the RMS: Based on the evaluation of the study the acute fish toxicity test is considered valid

Reference:	**RPA 400727- Acute toxicity (96 hours) to bluegill sunfish (*Lepomis macrochirus*) under flow-through conditions**
Author(s), year:	[redacted]., 1998b
Report/Doc. number:	R012019
Guideline(s):	E.P.A./FIFRA Guideline 72-1 (1985)
GLP:	Yes
Deviations:	Please refer to the commenting box below
Validity:	Acceptable

Material and methods:

Test substance:	RPA 400727, lot no: DA646, purity: 97.1%, water solubility limit approx. 9.2 mg/L
Test species:	Bluegill sunfish (*Lepomis macrochirus*)
Holding of fish :	Prior to the test, fish were held in a 500-L fiberglass tank under a photoperiod of 16 hours light and 8 hours dark. Through-flowing well water with total hardness of 28-30 mg/L, alkalinity of 22-24 mg/L, specific conductance range of 110 - 120 µS/cm, pH range of 6.7-6.9, dissolved oxygen concentration range of 68-76% of saturation, flow rate with 8.6-10.8 tank volume replacements/day. Fish used during definitive exposure were maintained under similar conditions for a minimum of 14 days prior to testing at a temperature range of 20-21°C.
Number of organisms:	2 replicates of 10 fish per concentration control and solvent control
Age, length, weight:	Representative sample (n=10) - Age: no information, mean total length: 37 mm (29-46), mean weight: 0.62 g (0.20-1.2)
Loading	0.0082 g biomass/L of flowing test solution per 24 hours.
Type of test:	Flow-trough, 96 hours

Applied concentrations:

Preliminary range finding test:	Concentrations ranging from 10.0-1.3 mg ai/L. Mortality of 10% was observed among fish exposed to the highest concentration tested.
Definitive test Nominal:	0 (control and solvent control), 1.3, 2.2, 3.6, 6.0, and 10 mg ai/L. The nominal concentration of 4.0 mg/l was above the visual limit of aqueous solubility. This was defined by the presence of undissolved particles in this test solution at test initiation.
Measured (mean):	0 (control and solvent control), measured concentrations 1.3, 2.0, 3.2, 5.4, 8.9 mg/L
Solvent:	Acetone, CAS no.:67-64-1

Test conditions:

Water quality:	Same well water than in the holding tank with total hardness of 26-30 mg/L, alkalinity of 20-22 mg/L.

Conductivity:	120-130 µS/cm
Temperature:	22-23°C
pH:	7.0-7.1
O_2 content:	7.2-8.7 mg/L (84-99% saturation)
Light regime:	Light/dark cycle of 16/8, light intensity: 323 lux
Feeding	Dry commercial pelleted food, ad *libitum*, daily except during the 48 hour prior to, and during the definitive test.
Methods:	Glass test aquariums 39 x 20 x 25 cm with a 14.5 cm high standpipe which maintained a constant test water volume of 11 L. The diluter was calibrated to deliver 500 ml/cycle of exposure solution to each replicate test aquarium which provided approx. 6.9 volume replacements per aquarium every 24 hours.
Test parameters:	Biological observations of the exposed bluegill sunfish and observations of the physical characteristics of the test solutions were made at test initiation and at each subsequent 24-hour interval until test termination after 96 hours. Mortalities were recorded and dead fish removed from each aquarium every 24 hours during the exposure period. Dissolved oxygen concentration, temperature and pH were measured once daily in each replicate of each treatment level and the controls throughout the exposure period. At test initiation, total hardness, alkalinity and specific conductance were measured in one replicate solution of the controls and each treatment level.
Analytical measurements:	Both replicates of the high, middle and low treatment levels and the dilution water control were sampled and analysed for RPA 400727 concentration prior to the start of the definitive exposure. During the in-life phase of the definitive study, water samples were removed from both replicate test solutions of each treatment level and the controls at 0 and 96 hours for analysis of RPA 400727 concentration.
Statistics:	No mortalities occurred in the treatments, therefore estimation of an LC_{50} was not possible.

Findings:

Analytical data:	Measured concentrations were 89-100% of the nominal concentrations. However, in the study report the results were based on mean measured concentrations.
Biological findings:	The preliminary test showed 10% mortality at the highest concentration tested, 10 mg ai/L. One of the fish exposed to the lowest concentration tested, 1.3 mg ai/L, exhibited complete loss of equilibrium. No mortalities were observed in the treatment groups and the control during the test period. The concentration of carrier solvent (acetone) was increased from 0.1 mL/L to approximately 0.5 mL/L in an effort to maximize the solubility of the test substance. Nevertheless, the presence of undissolved test material in the toxicant delivery system and in the exposure solution aquaria of the highest treatment level

was noted throughout the exposure and indicated that this test concentration (10 mg ai/L, nominal) exceeded the material's limit of water solubility.
5% mortality were observed in the solvent control group.

Conclusion: Based on mortality data, the LC_{50} was reported to be greater than the highest mean measured concentration of 8.9 mg/l after 96 hours of exposure.
Based on biological observations, the NOEC for rainbow trout is reported to be 8.9 mg/L.

Comment RMS: The study was evaluated following the recommendations of the currently valid test guidelines OPPTS 850.1075 (1996) and OECD 203 (1992).

Check of validity criteria:

OPPTS 850.1075 (1996):

- Maximum-allowable control or solvent control mortality is 10 percent (or 1 mortality if 7 to 10 control fish are used) for a 96-h period of testing. If the test is continued past 96 h, the maximum-allowable additional mortality is 10 percent. During the study 5% mortality were observed in the solvent control. Fulfilled.
- Constant conditions must be maintained throughout the test period. Flow-through procedures are preferred over static-renewal or semistatic procedures and static-renewal procedures are preferred over a static test procedure. The study was a flow-through test and constant conditons were maintained. Fulfilled.
- In flow-through test, the dissolved oxygen (DO) should be maintained above 75 percent saturation. The O_2-content in the study was 7.2-8.7 mg/L (84-99% saturation). Fulfilled.
- Measured concentrations are required if the test chemical is unstable or a flow-through diluter system is employed. In any case there must be evidence that test concentrations remained at least 80 percent of the nominal concentrations throughout the test or that mean measured concentrations are an accurate representation of exposure levels maintained throughout the test period. During the test, measured concentrations were 89-100% of the nominal concentrations. Fulfilled.

OECD 203 (1992):

- The mortality in the control(s) should not exceed 10 per cent (or one fish if less than ten are used) at the end of the test. During the study 5% mortality were observed in the solvent control. Fulfilled.

In addition, the following points deviated from the test guidelines or were not reported in detail:

- According to OPPTS 850.1075 (1996) the temperature should be tested hourly in one replicate throughout the test. The measurements in all replicates were

conducted at the start and every 24 hours throughout the exposure period.
The test substance should be measured in each replicate at 0, 48, and 96 h but chemical analysis was only conducted at test initiation and 24 hours after test termination. However the concentrations remained at 89 to 100% of the nominal concentrations.
- According to the OECD 203 (1992) the fish should have a total length of 2±1 cm but the fish used were 2.9-4.6 cm long.
Acceptability of the analytical methods used in the test: Evaluation of the analytical methods (water/sediment/soil) according to the Guidance Document SANCO/3029/99 rev. 4.
Method: HPLC/UV
Linearity: Calibration is based on duplicate determination at 5 concentrations.
The calibration function was linear within the range from 0.500-7.00 mg/L with r^2 > 0.993240.
Accuracy: 3 fortification levels (each 3 measurements): 15.0 mg/L, 7.00 mg/L and 0.500 mg/L.
Mean recoveries for each level: 104%, 106% and 101%
Precision: The relative standard deviation per fortification level was ≤ 20 %
LOQ: 0.2493 mg/L
LOD: -
The analytical method fulfils the requirements of guideline SANCO/3029/99 rev. 4.
Endpoints:
The RMS agrees on the endpoints given in the study report.
LC_{50} > 8.9 mg/L mean measured concentration
NOEC = 8.9 mg/L mean measured concentration
Conclusion of the RMS: Based on the evaluation of the study the acute fish toxicity test is considered valid.

Reference:	**BAS 595 F Acute Toxicity Study on the Rainbow Trout (*Oncorhynchus mykiss*) in a static system over 96 hours**
Author(s), year:	[redacted] 2006a
Report/Doc. number:	BASF DocID 2006/1015993
Guideline(s):	OECD 203; EPA-§ 72-1; OPPTS 850.1075
GLP:	Yes
Deviations:	Please refer to commenting box below
Validity:	Acceptable

Material and methods:

Test substance:	BAS 595 F; Batch: COD-000601; purity: 90.3%
Test species:	Rainbow trout (*Oncorhynchus mykiss*)
Animal supplier:	Forellenzucht Trostadt GbR, Germany
Acclimatization:	Acclimatization to test conditions including test water and light regime for 14 days.
Number of organisms:	10 individuals per test vessel, 1 test vessel per concentration
Age, length, weight:	Age: approx. 8 month, body length: 7.1 cm (5.7-8.3), body weight: 3.39 g (1.55-4.88)
Loading	0.7 g fish/L water
Type of test:	Static, 96 hours
Applied concentrations:	
Range finding:	The test concentrations were selected on the basis of a non-GLP range finding part of the study
Definitive test Concentrations:	0, 100, 50, 22, 10, 5 % saturated solution 0, 12.4, 6.25, 2.62, 1.18, 0.58 mg/L based on mean measured concentration of the technical test substance
Solvent:	none
Test conditions:	
Water quality:	Non chlorinated charcoal filtered tap water mixed with deionized water, aerated
Total hardness:	Approx 1 mmol/L = 100 mg/L $CaCO_3$
Conductivity:	250 µS/cm (at 25°C)
Temperature:	12-13°C
pH:	7.6-8.3
Oxygen content:	7.6-8.2 mg/L
Light regime:	Light/dark cycle of 16/8, light intensity: approx. 36-191 Lux
Feeding	Ecotstart 17 (Bio Mar) ad libitum, additionally generally on workdays live brine shrimp. Withdrawal of feed during the last day before start of

exposure.

Methods: Glass aquaria with stainless steel frame (60 cm long, 35 cm wide, 40 cm high) with 50 L volume

Test parameters: All test vessels were monitored for effects within 1 hour after start of exposure and 4, 24, 48, 72 and 96 hours after start of exposure.
Temperature, oxygen content and pH-value were measured in each test vessel within 1 hour after start of exposure and after 24, 48, 72 and 96 hours.

Analytical measurements: Samples for analytical determination of the concentration were taken within 30 minutes before insertion of the test organisms, after 48 hours and at the end of the exposure after approx. 96 hours. The transport to the analytical laboratory was done on the day of sampling. The analytical method was APL0500/02 HPLC-method with MS-detection and external calibration.

Statistics: No statistical analysis was carried out since no mortality was observed up to the highest tested concentration.

Findings:

Analytical data: Measured concentrations were 98.1-102% of the initial concentrations. In the study report the results were based on mean measured concentrations. All test batches were present as clear solutions over the exposure period according to visual inspection.

Biological findings: No mortality or significant sublethal toxic effects were observed in the dilution water control and solvent control groups during the test period.
In the two highest test concentrations (6.25 and 12.4 mg/L) one fish each died after 72 hours. Also sublethal effects (swimming at the bottom) were observed at these concentrations.

Table 9.2-1 Cumulative mortality data for rainbow trout exposed for 96 hours to BAS 595 F

Test concentration [mg ai/L]		**Cumulative mortality [%] (no. of dead fish / no. of treated fish)**					
		1 h	**4 h**	**24 h**	**48 h**	**72 h**	**96 h**
Control	Replicate 1	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)
	Replicate 2	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)
0.58	Replicate 1	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)
	Replicate 2	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)
1.18	Replicate 1	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)
	Replicate 2	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)
2.62	Replicate 1	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)
	Replicate 2	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)
6.25	Replicate 1	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)
	Replicate 2	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	10 (1/10)	10 (1/10)
12.4	Replicate 1	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	10 (1/10)	10 (1/10)

	Replicate 2	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)

Conclusion: Based on mortality data, the LC_{50} was reported to be greater than the highest mean measured concentration of 12.4 mg/l after 96 hours of exposure.
Based on biological observations, the NOEC to rainbow trout is reported to be 2.62 mg/L.

Comment RMS: The study was evaluated following the recommendations of the currently valid test guidelines OPPTS 850.1075 (1996) and OECD 203 (1992).

Check of validity criteria:

OPPTS 850.1075 (1996):

- Maximum-allowable control or solvent control mortality is 10 percent (or 1 mortality if 7 to 10 control fish are used) for a 96-h period of testing. If the test is continued past 96 h, the maximum-allowable additional mortality is 10 percent. During the study mortality occurred neither in the control nor in the solvent control. Fulfilled.
- Constant conditions must be maintained throughout the test period. Flow-through procedures are preferred over static-renewal or semistatic procedures and static-renewal procedures are preferred over a static test procedure. The study was a static test and constant conditons were maintained. Fulfilled.
- In static test, the dissolved oxygen (DO) in each replicate should at all times be greater than 60 percent saturation. The O2-content in the study was 7.6-8.2 mg/L (72.9-80.4% saturation). Fulfilled.
- Measured concentrations are required if the test chemical is unstable or a flow-through diluter system is employed. In any case there must be evidence that test concentrations remained at least 80 percent of the nominal concentrations throughout the test or that mean measured concentrations are an accurate representation of exposure levels maintained throughout the test period. During the test, measured concentrations were 98.1-102% of the initial concentrations. Fulfilled.

OECD 203 (1992):

- The mortality in the control(s) should not exceed 10 per cent (or one fish if less than ten are used) at the end of the test. During the study mortality occurred neither in the control nor in the solvent control. Fulfilled.

In addition, the following points deviated from the test guidelines or were not reported in detail:

- According to OPPTS 850.1075 (1996) the fish should not be fed for 48 hours

	before test initiation. The report states that the fish were not fed for at least 24 hours before test initiation. - According to OPPTS 850.1075 (1996) the temperature should be tested hourly in at least one replicate throughout the test. It was tested within 1 hour after start of exposure and after 24, 48, 72 and 96 hours. - According to the OECD 203 (1992) the fish should have a total length of 5±1 cm but the fish used were 5.7-8.3 cm long. The temperature range should be 13-17 °C but the temperature was most of the time below 13 °C. However it was constant within in a range of 2 °C. In the study report it is stated that one test vessel/concentration was used. However the result-tables show two replicates per test concentration. It is assumed that the statement of 1 test vessel/concentration is a typo. **Acceptability of the analytical methods used in the test:** Based on the evaluation (see Volume 3 B.5-CA, B.5.1.2.6) the analytical method is considered acceptable covering the LOQ of 0.001 mg ai/L. **Endpoints:** The RMS agrees on the endpoints given in the study report. LC_{50} > 12.4 mg/L mean measured concentration NOEC = 2.62 mg/L mean measured concentration **Conclusion of the RMS:** Based on the evaluation of the study the acute fish toxicity test is considered valid.

Reference:	**BAS 595 F Acute Toxicity Study on the Bluegill sunfish (*Lepomis macrochirus)* in a static system over 96 hours**
Author(s), year:	[redacted] 2006b
Report/Doc. number:	BASF DocID 2006/1018146
Guideline(s):	OECD 203; EPA-§ 72-1; OPPTS 850.1075
GLP:	Yes
Deviations:	Please refer to commenting box below
Validity:	Acceptable

Material and methods:

Test substance:	BAS 595 F; Batch: COD-000601; purity: 90.3%
Test species:	Bluegill Sunfish (*Lepomis macrochirus*)
Animal supplier:	Osage Catfisheries Inc, USA
Acclimatization:	Acclimatization to test conditions including test water and light regime for 14 days.
Number of organisms:	10 individuals per test vessel, 2 test vessels per concentration
Age, length, weight:	Age: approx. 12 month, body length: 4.5 cm (4.2-5.1), body weight: 1.2 g (0.94-2.01)
Loading	0.2 g fish/L water
Type of test:	static
Applied concentrations:	
Range finding:	The test concentrations were selected on the basis of a non-GLP range finding part of the study
Definitive test Concentrations:	0, 100, 50, 22, 10, 5 % saturated solution 0, 10.1, 5.10, 2.23, 1.01, 0.52 mg/L based on mean measured concentration of the technical test substance
Solvent:	none
Test conditions:	
Water quality:	Non chlorinated charcoal filtered tap water mixed with deionized water, aerated
Total hardness:	Approx 1 mmol/L = 100 mg/L $CaCO_3$
Conductivity:	250 µS/cm (at 25°C)
Temperature:	21.8-22.4°C
pH:	7.7-8.3
Oxygen content:	6.0-8.9 mg/L
Light regime:	Light/dark cycle of 16/8, light intensity: approx. 36-191 Lux
Feeding	Ecotstart 17 (Bio Mar) ad libitum, additionally generally on workdays live brine shrimp. Withdrawal of feed during the last day before start of exposure.
Methods:	Glass aquaria with stainless steel frame (60 cm long, 35 cm wide, 40 cm high)

	with 50 L volume
Test parameters:	All test vessels were monitored for effects within 1 hour after start of exposure and 4, 24, 48, 72 and 96 hours after start of exposure. Temperature, oxygen content and pH-value were measured in each test vessel within 1 hour after start of exposure and after 24, 48, 72 and 96 hours.
Analytical measurements:	Samples for analytical determination of the concentration were taken within 30 minutes before insertion of the test organisms, after 48 hours and at the end of the exposure after approx. 96 hours. The transport to the Analytical laboratory was done on the day of sampling. The analytical method was APL0500/02 HPLC-method with MS-detection and external calibration.
Statistics:	No statistical analysis was carried out since no mortality was observed up to the highest tested concentration.
Findings:	
Analytical data:	Measured concentrations were 95.2-108% of the initial concentrations. In the study report the results were based on mean measured concentrations. For the solubility behaviour of the test substance no remarkable observations were made
Biological findings:	No mortality or significant sublethal toxic effects were observed in the dilution water control, solvent control groups and the treatment groups during the test period.

Conclusion: Based on mortality data, the LC_{50} was reported to be greater than the highest mean measured concentration of 10.1 mg/l after 96 hours of exposure.
Based on biological observations, the NOEC to bluegill sunfish is reported to be 10.1 mg/L.

Comment RMS:	The study was evaluated following the recommendations of the currently valid test guidelines OPPTS 850.1075 (1996) and OECD 203 (1992). **Check of validity criteria:** OPPTS 850.1075 (1996): - Maximum-allowable control or solvent control mortality is 10 percent (or 1 mortality if 7 to 10 control fish are used) for a 96-h period of testing. If the test is continued past 96 h, the maximum-allowable additional mortality is 10 percent. During the study mortality occurred neither in the control nor in the solvent control. Fulfilled. - Constant conditions must be maintained throughout the test period. Flow-through procedures are preferred over static-renewal or semistatic procedures and static-renewal procedures are preferred over a static test procedure. The study was a static test and constant conditions were maintained. Fulfilled.

- In static test, the dissolved oxygen (DO) in each replicate should at all times be greater than 60 percent saturation. The O_2-content in the study was 6.0-8.9 mg/L (70.3-104.3% saturation). Fulfilled.
- Measured concentrations are required if the test chemical is unstable or a flow-through diluter system is employed. In any case there must be evidence that test concentrations remained at least 80 percent of the nominal concentrations throughout the test or that mean measured concentrations are an accurate representation of exposure levels maintained throughout the test period. During the test, measured concentrations were 95.2-108% of the initial concentrations. Fulfilled.

OECD 203 (1992):
- The mortality in the control(s) should not exceed 10 per cent (or one fish if less than ten are used) at the end of the test. During the study mortality occurred neither in the control nor in the solvent control. Fulfilled.

In addition, the following points deviated from the test guidelines or were not reported in detail:
- According to OPPTS 850.1075 (1996) the fish should not be fed for 48 hours before test initiation. The report states that the fish were not fed for at least 24 hours before test initiation.
- According to OPPTS 850.1075 (1996) the temperature should be tested hourly in at least one replicate throughout the test. It was tested within 1 hour after start of exposure and after 24, 48, 72 and 96 hours.
- According to the OECD 203 (1992) the fish should have a total length of 2±1 cm but the fish used were 4.2-5.1 cm long.

Acceptability of the analytical methods used in the test: The evaluation of the analytical methods was conducted according to the Guidance Document SANCO/3029/99 rev. 4. Based on the evaluation (see Volume 3 B.5-CA, B.5.1.2.6) it could be demonstrated that the method fulfils the requirements with regard to linearity, specificity, accuracy, repeatability, and limit of quantification. LOQ: 0.001 mg ai/L.

Endpoints:
The RMS agrees on the endpoints given in the study report.
LC_{50} > 10.1 mg/L mean measured concentration
NOEC = 10.1 mg/L mean measured concentration

Conclusion of the RMS: Based on the evaluation of the study the acute fish toxicity test is considered valid.

Reference:	**Triticonazole technical - Acute toxicity to Sheepshead Minnow (*Cyprinodon variegatus*) under flow-through conditions**
Author(s), year:	[redacted] 1998a
Report/Doc. number:	R000095
Guideline(s):	FIFRA Guideline 72-3
GLP:	Yes
Deviations:	Please also refer to commenting box below
Validity:	Acceptable

Material and methods:

Test substance:	Triticonazole technical (RPA 400727); Batch: OP9750057; purity: 90.52%
Test species:	Sheepshead Minnow (*Cyprinodon variegatus*)
Animal supplier:	Aquatic Biosystems, Colorado, USA
Acclimatization:	Acclimatization to test conditions including test water (pH 7.4 and 78-100% oxygen saturation) and light regime for 14 days. Temperature ranged from 21 to 22°C during this time.
Number of organisms:	10 individuals per test vessel, 2 test vessels per concentration
Age, length, weight:	Age: not stated, juvenile according to the study protocol; body length: 3.5 cm (3.0-4.0), body weight: 0.74 g (0.48-0.98)
Loading	0.1 g of biomass/L water
Type of test:	Flow-through, 96 hours

Applied concentrations:

Range finding:	The test concentrations were selected on the basis of preliminary testing
Nominal test Concentrations:	0, 1.3, 2.1, 3.6, 5.9 and 9.9 mg ai/L
Mean measured concentration	0, 1.2, 2.0, 3.4, 5.7, 9.1 mg/L
Solvent:	acetone

Test conditions:

Water quality:	Filtered seawater
Total hardness:	< 2 mg/L total organic carbon concentration
Salinity:	32‰
Temperature:	22-23°C
pH:	7.7-7.8
Oxygen content:	76-105% saturation
Light regime:	Light/dark cycle of 16/8, light intensity: 220-1100 Lux
Feeding	Commercial flaked food, *ad libitum*, daily. Fish were not fed during the 48-hour period prior to test initiation or during the exposure period.
Methods:	The exposure system consisted of an intermittent-flow proportional diluter with a 60% dilution factor, a temperature-controlled water bath and a set of fourteen exposure vessels. The exposure system was entirely constructed of

	glass and silicone sealant. Test solutions were not aerated. Each glass exposure aquarium measured 39 x 20 x 25 cm with a 14.5 cm high side drain which maintained a constant test solution volume of 11L. The flow of exposure solution to each aquarium was 0.5 L per minute which provided approximately 6.4 solution volume replacements per day in order to provide a 90% test solution replacement rate of approximately 8.5 hours. The concentration of acetone in the solvent control exposure vessels (0.53 mL/L) was equivalent to the concentration of solvent present in the highest treatment level solutions.
Test parameters:	All test vessels were monitored for effects at 0 and after 24, 48, 72 and 96 hours of exposure. Temperature, dissolved oxygen, pH-value and salinity were measured in each test vessel once a day throughout the exposure period. In addition, test solution temperature was continuously monitored in the control (replicate A) test vessel.
Analytical measurements:	During the in-life phase of the definitive study, one sample from each replicate of each treatment level and the control solutions was analysed for Triticonazole concentration at test initiation and 96 hours (test termination). In addition, three quality control (QC) samples were prepared at each sampling interval. The analytical method was HPLC-procedure based on methodology validated at the testing facility.
Statistics:	No statistical analysis was carried out since no lethality was observed up to the highest tested concentration.
Findings:	
Analytical data:	Measured concentrations were 92-97% of the nominal concentrations. In the study report the results were based on mean measured concentrations.
Biological findings:	No mortality or significant sublethal toxic effects were observed in the dilution water control and solvent control groups. At the highest test concentration of 9.1 mg ai/L all individuals were lethargic. At this concentration undissolved test substance was observed.
Conclusion:	Based on mortality data, the LC_{50} was reported to be greater than the highest mean measured concentration of 9.1 mg/l after 96 hours of exposure. Based on biological observations, the NOEC to sheepshead minnow is reported to be 5.7 mg/L.
Comment RMS:	The study was evaluated following the recommendations of the currently valid test guidelines OPPTS 850.1075 (1996) and OECD 203 (1992). **Check of validity criteria:**

OPPTS 850.1075 (1996):

- Maximum-allowable control or solvent control mortality is 10 percent (or 1 mortality if 7 to 10 control fish are used) for a 96-h period of testing. If the test is continued past 96 h, the maximum-allowable additional mortality is 10 percent. During the study mortality occurred neither in the control nor in the solvent control. Fulfilled.
- Constant conditions must be maintained throughout the test period. Flow-through procedures are preferred over static-renewal or semistatic procedures and static-renewal procedures are preferred over a static test procedure. The study was a flow-through test and constant conditons were maintained. Fulfilled.
- In flow-through tests, the dissolved oxygen (DO) should at all times be maintained above 75 percent saturation. The O_2-content in the study was 76-105% saturation. Fulfilled.
- Measured concentrations are required if the test chemical is unstable or a flow-through diluter system is employed. In any case there must be evidence that test concentrations remained at least 80 percent of the nominal concentrations throughout the test or that mean measured concentrations are an accurate representation of exposure levels maintained throughout the test period. During the test, measured concentrations were 92-97% of the nominal concentrations. Fulfilled.

OECD 203 (1992):

- The mortality in the control(s) should not exceed 10 per cent (or one fish if less than ten are used) at the end of the test. During the study mortality occurred neither in the control nor in the solvent control. Fulfilled

In addition, the following points deviated from the test guidelines or were not reported in detail:

- According to OPPTS 850.1075 (1996) the test substance should be measured in each replicate at 0, 48, and 96 h but chemical analysis was only conducted at test initiation and 24 hours after test termination. However the concentrations remained at 92 to 97% of the nominal concentrations.

Acceptability of the analytical methods used in the test: The evaluation of the analytical methods was conducted according to the Guidance Document SANCO/3029/99 rev. 4. Based on the evaluation (see Volume 3 B.5-CA, B.5.1.2.6) the analytical method is considered valid to quantify the amount of triticonazole in fresh and seawater.

LOQ: 0.148 mg/L

Endpoints:

The RMS agrees on the endpoints given in the study report.

	LC_{50} > 9.1 mg/L mean measured concentration NOEC = 5.7 mg/L mean measured concentration **Conclusion of the RMS:** Based on the evaluation of the study the acute fish toxicity test is considered valid.

Reference:	**BAS 595 F (Triticonazole) Acute Toxicity Study in the Common Carp (*Cyprinus carpio*)**
Author(s), year:	[redacted] 2014a
Report/Doc. number:	BASF DocID 2014/1095638
Guideline(s):	OECD 203; EPA-§ 72-1; OPPTS 850.1075
GLP:	Yes
Deviations:	Please refer to the commenting box below
Validity:	Acceptable

Material and methods:

Test substance:	BAS 595 F (Triticonazole); Batch: COD-001440; purity: 91.3%
Test species:	Common Carp (*Cyprinus carpio*)
Animal supplier:	Fischzucht Rhönforelle GmBH & Co. KG, Germany
Acclimatization:	Acclimatization to test conditions including test water and light regime for 14 days.
Number of organisms:	10 individuals per test vessel, 2 test vessels per concentration
Age, length, weight:	Age: 3 month; body length: 4.1 cm (3.7-4.5), body weight: 0.82 g (0.54-1.09)
Loading	0.21 g fish/L water
Type of test:	Static, 96 hours
Applied concentrations:	
Nominal test Concentrations:	0, 6.25, 12.5, 25, 50, 100% as saturated solution
Mean measured concentration	0, 1.1, 2.2, 4.4, 9.1 and 18 mg/L
Solvent:	none
Test conditions:	
Water quality:	Charcoal-filtered drinking water from the municipal water works of the city of Frankenthal mixed with deionised water
Total hardness:	100 $CaCO_3$ mg/L
Temperature:	21.8-22.1°C
pH:	8.1-8.4
Oxygen content:	6.1-8.4 (> 60% saturation)
Light regime:	Light/dark cycle of 16/8, light intensity: 60-585 Lux
Feeding	Inicio 917 (Bio Mar), *ad libitum*. Fish were not fed during the 48-hour period prior to test initiation.

Methods: Stainless steel aquaria, approx. 50 L (60 x 35 x 40 cm); test volume: 40 L

Test parameters: All test vessels were monitored for effects within 1 hour after start of exposure and 6, 24, 48, 72 and 96 hours after start of exposure.
Temperature, dissolved oxygen and pH-value were measured in each test vessel within 1 hour after start of exposure and after 24, 48, 72 and 96 hours. In addition, temperature was continuously monitored in one control vessel.

Analytical measurements: Sampling for concentration control analysis was done within 30 min before insertion of the test organisms after approx. 48 hours and 96 hours from all replicates per concentration. The samples for analysis were collected with a glass pipette inserted in the middle of the test vessel.
The analytical method was APL0500/03, HPLC with MS-detection and external calibration

Statistics: No statistical analysis was carried out since only one fish died in the highest tested concentration.

Findings:

Analytical data: Measured concentrations were 93-103% of the nominal concentrations. In the study report the results were based on mean measured concentrations. All test treatments were visibly clear over the entire exposure period and no undissolved material was observed.

Biological findings: No mortality or significant sublethal toxic effects were observed in the dilution water control group. At the highest test concentration of 18 mg ai/L one fish died. At this concentration 14 fish showed sublethal effects (tottering, swimming at the bottom).

Table 9.2-2 Cumulative mortality data for common carp exposed for 96 hours to BAS 595 F

Test concentration [mg ai/L]		**Cumulative mortality [%] (no. of dead fish / no. of treated fish)**					
		1 h	**6 h**	**24 h**	**48 h**	**72 h**	**96 h**
Control	Replicate 1	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)
	Replicate 2	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)
1.1	Replicate 1	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)
	Replicate 2	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)
2.2	Replicate 1	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)
	Replicate 2	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)
4.4	Replicate 1	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)
	Replicate 2	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)
9.1	Replicate 1	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)
	Replicate 2	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)
18	Replicate 1	0 (0/10)	0 (0/10)	10 (1/10)	10 (1/10)	10 (1/10)	10 (1/10)
	Replicate 2	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)	0 (0/10)

Conclusion:	Based on mortality data, the LC_{50} was reported to be greater than the highest mean measured concentration of 18 mg/l after 96 hours of exposure. Based on biological observations, the NOEC to common carp is reported to be 9.1 mg/L.
Comment RMS:	The study was evaluated following the recommendations of the currently valid test guidelines OPPTS 850.1075 (1996) and OECD 203 (1992). **Check of validity criteria:** OPPTS 850.1075 (1996): - Maximum-allowable control or solvent control mortality is 10 percent (or 1 mortality if 7 to 10 control fish are used) for a 96-h period of testing. If the test is continued past 96 h, the maximum-allowable additional mortality is 10 percent. During the study mortality occurred neither in the control nor in the solvent control. Fulfilled. - Constant conditions must be maintained throughout the test period. Flow-through procedures are preferred over static-renewal or semistatic procedures and static-renewal procedures are preferred over a static test procedure. The study was a static test and constant conditons were maintained. Fulfilled. - In flow-through tests, the dissolved oxygen (DO) in each replicate should at all times be greater than 60 percent saturation. The O2-content in the study was 6.1-8.4 (71.5 - 98.5% saturation). Fulfilled. - Measured concentrations are required if the test chemical is unstable or a flow-through diluter system is employed. In any case there must be evidence that test concentrations remained at least 80 percent of the nominal concentrations throughout the test or that mean measured concentrations are an accurate representation of exposure levels maintained throughout the test period. During the test, measured concentrations were 93-103% of the nominal concentrations. Fulfilled. OECD 203 (1992): - The mortality in the control(s) should not exceed 10 per cent (or one fish if less than ten are used) at the end of the test. During the study mortality occurred neither in the control nor in the solvent control. Fulfilled. **In addition, the following points deviated from the test guidelines or were not reported in detail:** - According to the OECD 203 (1992) the fish should have a total length of 3±1 cm but the fish used were 3.7-4.5 cm of length. **Acceptability of the analytical methods used in the test:** The evaluation of the analytical methods was conducted according to the Guidance Document

	SANCO/3029/99 rev. 4. Based on the evaluation (see Volume 3 B.5-CA, B.5.1.2.6) it could be demonstrated that the method fulfils the requirements with regard to linearity, specificity, accuracy, repeatability, and limit of quantification. LOQ: 0.001 mg ai/L. **Endpoints:** The RMS agrees on the endpoints given in the study report. LC_{50} > 18 mg/L mean measured concentration NOEC = 9.1 mg/L mean measured concentration **Conclusion of the RMS:** Based on the evaluation of the study the acute fish toxicity test is considered valid.

Reference:	**Experimental assessment of the environmental fate and effects of triazoles and benzotriazloe**
Author(s), year:	Durjava, M.K. et al., 2013a
Report/Doc. number:	BASF DocID 2015/1177633
Guideline(s):	None
GLP:	No

Material and methods:

Test substance:	Triticonazole (BAS 595 F), CAS no.: 131983-72-7; purchased from Fluka® Analytical (Sigma-Aldrich).
Test species:	Zebrafish (*D. rerio*), embryos (viable eggs).
Test design:	Static system (72 h); each test was repeated three times; if necessary, the concentration range was adjusted between different experiments; viable eggs were incubated individually in 2 mL of medium in well flat-bottomed plates; 4 wells per plate were used for control, 10 wells for each test concentration; stock solutions were prepared in DMSO, with a final test concentration of 0.2% carrier (2 mL/L) in the dilution series; microscopic observations 24, 48 and 72 hours after start of the experiments.
Test concentrations:	6 triticonazole test concentrations (range was adjusted between different experiments if necessary), solvent: DMSO (2 ml/L), negative control: potassium dichromate (1 mg/L); positive control: 3,4-dichloroaniline (8 mg/L).
Test conditions:	Well flat-bottomed plates; 2 mL of medium per well; dilution water: Dutch Standard Water (DSW; demineralized water supplemented with 100 mg/L $NHCO_3$, 20 mg/L $KHCO_3$, 200 mg/L $CaC_{12}·2H_2O$ and 180 mg/L $MgSO_4·7H_2O$, aerated for 24 hours at 27°C); temperature: 26.5 ± 0.5°C;

	pH of dilution water: 8.1 (7.4 - 8.3); oxygen content: ≥ 6.6 mg/L; water hardness: 214 mg $CaCO_3$/L.
Test parameters:	LC_{50}, mortality, no heartbeat, no somite formation, no detachment of tail.
Analytics:	Chemical measurements of test item concentrations was conducted using a GC-method with MS detection
Statistics:	Descriptive statistics; trimmed Spearman-Karber method for calculation of LC_{50} values.

Findings:

Analytical data: Analytical measurements: Chemical measurements of test item concentrations in the test cultures was conducted at the beginning and at the end of the test. The following biological results are based on nominal concentrations.

Biological findings:

Table 9.2-3 Acute toxicity (72 h) of triticonazole to zebrafish (D. rerio)

		Endpoints [mg triticonazole/L] (nominal)
LC_{50} (72 h)	test 1	≥ 31.80 (95% confidence limits: n.a.)
	test 2	≥ 95.30 (95% confidence limits: n.a.)
	test 3	≥ 95.30 (95% confidence limits: n.a.)

n.a. = not applicable

Conclusion: In a static acute toxicity study with zebrafish (test repeated three times), the LC_{50} values (72 h) of triticonazole were ≥ 31.80 mg ai/L (test 1) and ≥ 95.30 mg ai/L (test 2 and 3) based on nominal concentrations.

Comment RMS:	The public literature study with zebra fish indicates that zebra fish is not more sensitive to triticonazole than other tested fish species.

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

For the first EU peer-review of the active substance triticonazole a 28 day, juvenile fish growth study was submitted addressing the chronic risk to fish. However, this study does not fulfil the new data requirements under Regulation 1107/2009. Thus, a new fish full life cycle study has been performed with the active substance triticonazole.

Reference:	**BAS 595 F (Triticonazol) - Life cycle test on the fathead minnow (*Pimephales promelas*) in a flow through system**
Author(s), year:	[redacted] 2008a
Report/Doc. number:	BASF Doc DocID 2008/1028361
Guideline(s):	(U.S.) EPA-FIFRA 72-5; OPPTS 850.1500, Public draft 1996
GLP:	Yes
Deviations:	Please refer to commenting box below
Validity:	Acceptable

Material and methods:

Test substance:	BAS 595 F (Triticonazol); CAS no. : 131983-72-7, batch no. : COD-000601, purity : 93.6%
Test species:	Freshly fertilized eggs of the fathead minnow (*Pimephales promelas*)
Holding of fish :	Parental fish were kept in groups of 1 male plus 2 females. No further information is given about the holding of the fish. Test-vessels for exposure of early life stages: 1.7 L volume Glass aquaria for juveniles and adults: 24 L volume Stainless steel aquaria for paired groups: 9 L volume Feeding: Adults were fed two times daily with commercial diet and larvae of *Artemia salina*.
Number of organisms:	F1-generation: 8 replicates per concentration group (the reserve group not used for pairing was sacrificed 5 days before the sacrifice of the pair groups. F2-generation: 4 replicates of 25 intact eggs per test concentration and controls
Age:	Freshly fertilized eggs, < 6 hours
Type of test:	Flow-through test (water flow rates ensured a daily volume exchange range of at least 5)

Applied concentrations:

Nominal:	0 (control), 0.003, 0.006, 0.012, 0.024, and 0.048 and mg ai/L
Measured (mean):	0 (control), 0.0029, 0.006, 0.0114, 0.0229 and 0.0462 mg ai/L
Solvent:	None (aceton was used for solution but was completely evaporated)

Test conditions:

Water quality:	Aerated non-chlorinated charcoal-filtered tap water mixed with deionized water prepared in the testing facility. The mixing ration was adjusted to receive water with a hardness of approximately 100 mg/ L $CaCO_3$
Temperature:	25 ± 1 °C measured F1-generation: 23.8 – 24.8 °C; values < 24 °C for 6 days and 18 hours measured F2-generation: 23.4 – 24.3 °C; values < 24 °C for 22 days and 5 hours
pH:	F1-generation: 7.5 – 7.8 F1-genereation (pair groups=: 7.6 – 8.0) F2-generation: 7.2-7.9
O_2 content:	F1-generation: 6.0-8.5 (min 72% saturation); aeration since study day 22 F1-generation (pair groups): 6.1-8.4 (min 73% saturation); aeration since study day 106 F2-generation: 6.5-10.1 (min 78% saturation); aeration since study day 145)
Light regime:	Light/dark cycle of 16/8 F1-generation: 1.7 L test vessels: 255-515 Lux, 24 L glass aquaria: 102-230 Lux F1-generation (pair groups): 9 L stainless steel aquaria: 87-257 Lux F2-generation: 1.7 L test vessels: 264-492 Lux, 24 L glass aquaria: 162- 419 Lux
Feeding	Newly hatched larvae received two times per day freshly hatched live brine shrimp nauplii (*Artemia salina*). The combination of live artemia and commercial food was fed increasing in quantitiy with the size of the fish. The food was applied ad libitum applied in sufficient quantity to achieve fast growth. Approx. 24 hours before sacrifice no feeding of the animals was performed.
Methods:	The in life phase was started with the introduction of fertilised eggs, not older than 6 hours. The eggs to start the F1-generation were taken from 14 different parent groups and were pooled before they were distributed randomly to the test groups. Eggs, larvae and juveniles were exposed in cylindrical glass vessels with a water volume of approx. 1.7 L. On day 18 of exposure the fish were transferred to 30 L glass aquaria, water volume approx. 24 L. Start of hatch was the time of hatch of the first viable larva in one of the concentration groups or the control group. Start of hatch in the F1-generation was day 2. Hatch was considered complete, if no further hatch was expected in one of the test groups, (95% of surviving embryos of control group hatched). On day 36 (30 days post hatch) the group size was reduced to 15 animals per test tank (replicate). From day 68 (62 days post hatch) on each aquarium was equipped with 3 spawning tiles. They were controlled once daily in the morning for eggs to determine the time span to maturation for each replicate. The F2-generation was exposed in the same way as the F1-generation in

cylindrical glass vessels until day 17 after egg insertion and the glass aquaria from then on until sacrifice. Start of hatch in the F2-generation was day 2 after insertion. The P-generation was sacrificed on day 61 after insertion of the eggs. All fish were measured for length and weight.

Test parameters: Mortalities of different life stages, hatching rates (P- and F_1 generation, respectively), juvenile and adult growth, spawning performance, fertilisation rate, and sex ratio were recorded.

All fish were observed daily for mortality and any other abnormalities in appearance and behaviour.

Analytical measurements: F1-generation: twice during the week before start of exposure, at start of exposure (day 0) and from then on once weekly alternating in one representative replicate per concentration group.

F2-generation: In each replicate of all test groups at the day of insertion of eggs, afterwards weekly, alternating in one replicate of each concentration group

The test item concentrations were analyzed using a HPLC/MS method.

Statistics: Descriptive statistics; one-sided Fisher´s exact test and one-sided Wilcoxon-test for survival; two-sided Dunnett`s test for growth data; one-sided Wilcoxon-Test for number of eggs/day and fertility and two-sided for number of clutches/day, respectively.

Findings:

Analytical data: Analytical measurements: The control groups were free of test substance contaminations over the whole exposure period. The mean analytically determined concentrations of the test substance in the test water were generally in the range of ± 20% of the nominal concentration, but some deviations occurred during the 6 months exposure period.

Values below 80% of the nominal concentration were observed on one occasion at the test item concentration of 0.012 mg ai/L in the F1-generation until reproduction and on one occasion during exposure of the F1-generation pair groups at the test concentrations of 0.003 and 0.048 mg ai/L. In the F2-generation values below 80% were determined during the first week of exposure and on one additional occasion in the test groups 0.012 and 0.048 mg ai/L and on two occasions in the test concentration of 0.024 mg ai/L. The lowest value determined in the concentration of 0.012 mg ai/L (= NOEC) was 65% of the nominal concentration over less than one day at the start of the F1-generation and 74% of nominal during the exposure of the F2-generation.

Deviations from the nominal concentration > 120% of the nominal concentration (up to 140%) were observed on 2 - 3 occasions during the exposure of the F1-

generation until formation of pair groups. During exposure of the pair groups a concentration of 0.276 mg ai/L (574% of nominal) was determined in a sample relevant for 4 of the 8 pair groups of the 0.048 mg ai/L group and 147% of nominal was determined for the other 4 replicates at the same time. It was concluded that these samples were contaminated and that the values were not actually that high, however, this assumption could not be proved by additional measurements. Since the concentration of 0.048 mg ai/L was above the LOEC, a possible peak exposure would not change the interpretation of the overall results of the study.

During exposure of the F2-generation the measured concentrations exceeded 120% of the nominal concentration on one occasion in the test concentrations 0.003, 0.006, 0.012 and 0.048 mg ai/L. The highest measured concentrations in 0.024 mg ai/L group were 139% of nominal during exposure of the F1-generation and 120% of nominal during exposure of the F2-generation.

The mean analytical values are presented in the following table. The effect values were evaluated based on mean measured test item concentrations.

Table 9.2-4: Mean analytically determined concentration for triticonazole during the 6 month FFLC study

mg/L (nominal)	F1-generation day 0-95	F1-generation day 95-131, pair groups	F2-generation day 0-61 after egg insertion
0	Not detectable	Not detectable	Not detectable
0.003	0.0030 ± 0.0004 mg/L (100.4%)	0.0027 ± 0.0002 mg/L (90.2%)	0.003 ± 0.00065 mg/L (99.5%)
0.006	0.0061 ± 0.001 mg/L (101.1%)	0.0058 ± 0.00023 mg/L (95.5%)	0.006 ± 0.00146 mg/L (99.7%)
0.012	0.0119 ± 0.002 mg/L (99.6%)	0.011 ± 0.00056 mg/L (91.4%)	0.0116 ± 0.00203 mg/L (96.6%)
0.024	0.0248 ± 0.0034 mg/L (103.2%)	0.0216 ± 0.00151 mg/L (89.9%)	0.0225 ± 0.00381 mg/L (93.8%)
0.048	0.0492 ± 0.0051 mg/L (102.5%)	0.0425 ± 0.00374 mg/L (88.6%)	0.0477 ± 0.00772 mg/L (99.5%)

Biological results

In the nominal test concentrations up to and including 0.012 mg ai/L (0.0114 mg ai/L mean measured) no treatment related effects on the test organisms were observed. In the tests group exposed to a nominal concentration of 0.024 mg ai/L (0.0229 mg ai/L mean measured concentration) the body length and weight of the fish of the F2-generation was statistically significantly decreased. In the test group exposed to a nominal concentration of 0.048 mg ai/L (0.0462 mg ai/L mean measured concentration) a statistically significantly decreased body length of the fish of the F2-generation was observed. However, the decrease was less pronounced than in the lower test concentration 0.024 mg/L and within the normal variability. Nevertheless, the effects on growth in both test groups (0.024

and 0.048 mg ai/L) were considered for the evaluation. In the 0.048 mg a.s/L group a slight delay of the time to maturity in the F1-generation could not be excluded. No test substance-related effects on survival of both generations, on the growth of the F1-generation or on the reproduction of the F1-generation (fertility and fecundity) were observed in any of the test groups. The results are summarized in Table 9.2.2-2.

Table 9.2-5: Chronic toxicity (FFLC, 6 months) of triticonazole on fathead minnow (Pimephales promelas)

Concentration [mg ai/L] nominal			**Control**	**0.003**	**0.006**	**0.012**	**0.024**	**0.048**
Concentration [mg ai/L] mean measured over all study parts			**Control**	**0.0029**	**0.006**	**0.0114**	**0.0229**	**0.0462**
Survival	F1	start - hatch [%]	84	86	87	91	89	89
		hatch - swim up [%]	92	94	87	90	90	90
		swim up - reduction [%]	97	95	99	96	98	98
		reduction - reproduction [%]	100	100	98	95	98	100
		start reproduction - sacrifice [%]	93	100	97	95	98	100
	F2	start - hatch [%]	94	93	94	95	97	96
		hatch - swim up [%]	91	95	93	95	93	95
		swim up - end [%]	90	94	98	90	93	92
Growth	F1	length on day 36 [cm]	2.774	2.771	2.682	2.601**	2.676	2.729
		(SD)	(0.333)	(0.333)	(0.359)	(0.416)	(0.337)	(0.295)
		deviation from control [%]	--	-0.1	-3.3	-6.2	-3.5	-1.6
		length on day 68 [cm]	4.427	4.822**	4.712*	4.606	4.629	4.662
		(SD)	(0.642)	(0.555)	(0.565)	(0.512)	(0.548)	(0.482)
		Deviation from control [%]	--	+8.9	+6.4	+4.1	+4.6	+5.3
		male length at sacrifice [cm] pairs	6.700	6.856	6.613	6.925	6.725	6.688
		(SD)	(0.283)	(0.381)	(0.412)	(0.167)	(0.271)	(0.340)
		female length at sacrifice [cm] pairs	5.388	5.729	5.713	5.671	5.613	5.538
		(SD)	(0.541)	(0.439)	(0.189)	(0.335)	(0.236)	(0.226)
		male weight at sacrifice [g] pairs	4.455	4.646	4.440	4.365	4.221	4.426
		(SD)	(0.514)	(1.043)	(0.834)	(0.350)	(0.541)	(0.750)
		female weight at sacrifice [g] pairs	2.095	2.334	2.238	2.320	2.095	2.018
		(SD)	(0.285)	(0.557)	(0.309)	(0.918)	(0.406)	(0.274)
	F2	length at day 35 [cm]	2.467	2.623**	2.546	2.529	2.478	2.657**
		(SD)	(0.272)	(0.237)	(0.224)	(0.241)	(0.264)	(0.216
		deviation from control [%]	--	+6.3	+3.2	+2.5	+0.5	+7.7
		length at end [cm]	4.522	4.441	4.420	4.428	**4.247****	**4.289****
		(SD)	(0.368)	(0.342)	(0.300)	(0.302	(0.294)	(0.279)

Concentration [mg ai/L] nominal			**Control**	**0.003**	**0.006**	**0.012**	**0.024**	**0.048**
Concentration [mg ai/L] mean measured over all study parts			**Control**	**0.0029**	**0.006**	**0.0114**	**0.0229**	**0.0462**
		deviation from control [%]	--	-1.8	-2.3	-2.1	-6.1	-5.2
		weight at end [g]	0.941	0.918	0.859	0.869	**0.764****	0.886
		(SD)	(0.234)	(0.235)	(0.200)	(0.200)	(0.184)	(0.209)
		deviation from control [%]	--	-2.4	-8.6	-7.7	-18.8	-5.8
Reproduction	F1	% fertility	98.6	98.4	98.3	99.0	99.4	98.3
		(SD)	(0.90)	(1.2)	(1.7)	(0.5)	(0.5)	(1.2)
		eggs/female/day	17.9	17.0	16.4	17.3	17.4	14.3
		(SD)	(10.1)	(17.8)	(19.13)	(12.3)	(16.9)	(10.1)
		clutches/female/day	0.225	0.171	0.175	0.175	0.133	0.217
		(SD)	(0.112)	(0.138)	(0.118)	(0.123)	(0.087)	(0.093)
Endpoints [mg ai/L]								
Overall NOEC (nominal)			0.012					
Overall NOEC (mean measured)			0.0114					

* Statistically significant differences compared to the control ($p \leq 0.05$)
** Statistically significant differences compared to the control ($p \leq 0.01$)
Deviations which are considered to be substance-related are printed **bold**
SD Standard Deviation

Conclusion: The overall-NOEC observed in this study is 0.0114 mg ai/ and the respective overall LOEC is 0.0229 mg ai/L mean measured concentration.

Comment RMS: The study was evaluated following the recommendations of the currently valid test guidelines OPPTS 850.1500, Public draft 1996, OECD 210 (2013) and OECD Detailed Review Paper on Fish Life-Cycle Tests No. 95

Check of validity criteria:

OPPTS 850.100 (Public draft, 1996) and OECD Detailed Review Paper on Fish Life-Cycle Tests No. 95:

No validity criteria stated in these documents

OECD 210 (2013):

- The dissolved oxygen concentration should be > 60% of the air saturation value throughout the test. The dissolved oxygen concentration was between 72% and 103% of the air saturation. Fulfilled
- The water temperature should not differ by more than ±1.5°C between test chambers or between successive days at any time during the test, and should be within the temperature ranges of 25 ± 1.5°C for *Pimephales promelas.* The temperatures were between 23.4 and 25.5 °C.
- The analytical measure of the test concentrations is compulsory. Fulfilled.

- Overall survival of fertilised eggs and post-hatch success in the controls and, where relevant, in the solvent controls should be greater than or equal to 70 and 75%, respectively for *Pimephales promelas*. The fertility rate was 98.6 % and the post-hatch success 92%. Fulfilled.

In addition, the following points deviated from the test guidelines or were not reported in detail:

- Reduction of the group size occurred on day 30 to 15 animals per replicate. The possibility of a thinning event is mentioned in OPPTS 850.1400 – Fish Early Life Stage Toxicity Test, but not in OPPTS 850.1500 and OECD 210.
- Deviations from the nominal concentration > 120% of the nominal concentration (up to 140%) were observed on 2 - 3 occasions during the exposure of the F1-generation until formation of pair groups. During exposure of the pair groups a concentration of 0.276 mg ai/L (574% of nominal) was determined in a sample relevant for 4 of the 8 pair groups of the 0.048 mg ai/L group and 147% of nominal was determined for the other 4 replicates at the same time. It was concluded that these samples were contaminated and that the values were not actually that high, however, this assumption could not be proved by additional measurements. Since the concentration of 0.048 mg ai/L was above the LOEC, a possible peak exposure would not change the interpretation of the overall results of the study.

Acceptability of the analytical methods used in the test: The evaluation of the analytical methods was conducted according to the Guidance Document SANCO/3029/99 rev. 4. Based on the evaluation (see Volume 3 B.5-CA, B.5.1.2.6) it could be demonstrated that the method fulfils the requirements with regard to linearity, specificity, accuracy, repeatability, and limit of quantification.
LOQ: 0.001 mg ai/L.

Endpoints:

The RMS agrees on the endpoints given in the study report. ECx-values were not provided with the study report. Analysis with Tox RatPro® v.3.2 showed that no EC_x values could be determined for replicate means of larval length and weight and replicate proportions of hatching success/embryo survival, and fry survival, respectively.

NOEC = 0.0114 mg ai/L mean measured concentration

LOEC = 0.0229 mg ai/L mean measured concentration

Conclusion of the RMS: Based on the evaluation of the study the fish full life cycle test is considered valid.

Reference:	**BAS 595 F (Triticonazol) - Life cycle toxicity test on the fathead minnow (*Pimephales promelas*) in a flow through system**
Author(s), year:	[redacted] 2012a
Report/Doc. number:	BASF DocID 2012/1079000
Guideline(s):	(U.S.) EPA-FIFRA 72-5; OPPTS 850.1500; OECD (2008) Detailed Review Paper on Fish Life-cycle Tests
GLP:	Yes
Deviations:	Please refer to the commenting box below
Validity:	Acceptable

Material and methods:

Test substance:	BAS 595 F (Triticonazol); CAS no. : 131983-72-7, batch no. : COD-001440, purity : 91.3%
Test species:	Freshly fertilized eggs of the fathead minnow (*Pimephales promelas*)
Holding of fish :	Parental fish were kept flow-through glass aquaria (45-L) divided into three spawning groups consisting of either 1 male and 2 females or 2 males and 3 females. Mature fish were sexed according to external secondary sexual characteristics. A total of 33 spawning groups were used to produce eggs to initiate the exposure. Exposure of the F0-generation was started within 7 hours after fertilization. The egg stage was confirmed by examining 10 representative eggs under a stereo microscope. All embryos were in the blastula stage. Test-vessels for embryo/larval exposure: 1.7 L volume Stainless steel aquaria for juvenile exposure: 9 L volume Glass aquaria for juveniles and adults: 45 L volume
Number of organisms:	F0 embryo groups until end of hatch: 4 replicates with 50 fertilized eggs each F0 larval-juvenile groups until 8 weeks post-hatch: 4 replicates with 25 larvae each F0 juvenile-adult groups until maturity (day 60): 2 replicates with 25 juvenile fish each F0 reproduction groups after maturity: 4 replicates with 3 female and 2 male fish each F1 embryo groups until end of hatch: 2 replicates with 50 eggs each F1 larval-juvenile groups until sacrifice: 2 replicates with 25 larvae each
Age:	Freshly fertilized eggs, < 7 hours
Type of test:	Flow-through test (water flow rates ensured a daily volume exchange range of 4- to 5-fold volume exchange)

Applied concentrations:

Nominal:	0 (control), 0.006, 0.012, 0.024, 0.048, and 0.096 and mg ai/L

Time weighted Mean (mean):	0 (control), 0.007, 0.0125, 0.0238, 0.0473 and 0.0937 mg ai/L
Solvent:	None
Test conditions:	
Water quality:	Aerated non-chlorinated charcoal-filtered drinking water mixed with deionized water to achieve a hardness of approx. 100 mg/L $CaCO_3$.
Temperature:	F0 generation (days 0-60): 23.5-26 °C F0 generation (days 60-257): 24.5-26°C F1 generation (days 153-215): 24.9 – 26 °C
pH:	F0-generation: 7.6 – 8.2 F1-genereation: 7.7 – 8.4
O_2 content:	5.8-8.3 µg/L, approx. 69-99% of the maximum saturation at test temperature of 25°C; slight aeration was administered for F0-generation from day 7 and for the F1 generation from test day 161 (8 days after insertion) on.
Light regime:	Light/dark cycle of 16/8 – by day 206 the day length photoperiod was reduced by 30 minutes per week for the spawning F0 groups light intensity: 28-151 Lux
Feeding	Initiated for F0 and F1-generation in all test groups at the end of hatch (day 5) with live brine shrimp nauplii (*Artemia sp*.). Feeding was conducted at least twice daily on workdays and once or twice daily on non-working days. The combination of live *Artemia* and commercial food was fed in increasing quantitiy with the size of the fish and was continued until one day before the termination of exposure.
Methods:	*F0-Embryo groups:* 200 eggs (embryos not older than 7 hours) were inserted per test group. Start of hatch was defined as the day before the first viable larva was seen during the daily observation. The end of hatch was defined as the day on which the last newly hatched larva in one of the test groups was observed. F0-Laval-juvenile groups: When hatch was complete the replicate size was impartially reduced to 25 animals and the larvae were transferred to 9-L aquaria All groups were reduced on the same day. The remaining individuals were sacrificed. *F0-Juvenile-adult groups:* On test day 60 (55 days post hatch), the number of live fish was impartially reduced to 50 per test group, with 25 fish in each of two replicates in 45-L glass aquaria. The remaining fish were sacrificed. On test day 77, 4 spawning tiles were placed in each test vessel. On day 143 when at least four males and six females per replicate could be clearly distinguished by phenotype, they were separated into reproduction groups for the remainder of exposure. Of the remaining animals, some were selected as reserve fish and the rest of the F0-fish were sacrificed.

F0-Reproduction groups: Each replicate test aquaria was divided into three compartments using a coated stainless steel mesh basket. Two compartments had spawning tiles and housed reproduction groups of 2 male and 3 female fish. Reserve fish were kept in the mesh baskets. Two spawning tiles were placed into each spawning compartment.

F1-generation: consisted of 2 replicates of 50 intact embryos. All test groups were initiated on test day 153 (F1 day =). Eggs were pooled from 3-5 clutches originating from each corresponding F0-generation reproduction test group. Larval fish were impartially reduced to 25 per replicate and transferred to the larger test vessels on F1 day 5. The juveniles of the F2-generation were sacrificed 8 weeks after hatch on test day 215 (F1 day 62).

Test parameters:

F0-Embryo groups: From the first day of exposure until hatching was complete, embryos were observed daily for hatching and mortality. During the hatch the numbers of live and dead larvae and live and dead embryos were determined daily.

F0-Larval-juvenile groups: the numbers of dead, alive and deformed larvae were recorded during the reduction. After reduction dead test organisms were counted and removed daily and all surviving fish were counted at least once weekly. Observations of signs of effect and abnormalities were performed daily as soon as the size of the organisms enabled an observation (day 14). The standard length of all fish was determined by photography on test days 31 and 60 (approx. 4 and 8 week old larvae).

F0-Juvenile-adult groups: Of all the sacrificed fish body length and wet weight were measured and any clear signs of sexual maturity or obvious deformities were noted. The spawning tiles were examined once daily in the morning for eggs.

F0-Reproduction groups: The spawning tiles were examined daily. The number of eggs in all reproduction groups were counted and removed from the tiles daily.

Fertility was evaluated in the reproduction groups for at least the first 6 clutches (≥ 10 eggs) by counting fertilized and unfertilized eggs and expressed as % fertile eggs.

F1-generation: The length was determined by photography 4 weeks after hatch (day 33) and the total body length and the body weight were determined at sacrifice. Any observed malformations were documented.

Analytical measurements:

Concentration control samples were collected at start of exposure (test day 0), before insertion of fertilized eggs and then once weekly to determine concentrations of the test substance in the test vessels. Samples were collected from one alternating replicate per test group. On two occasions (days 12 and 180), samples were collected from all test group replicates of the F0 generation to confirm the uniformity of the concentration within each test group. Additional

samples were collected in those cases when deviation > 20 % from the nominal values were obtained to confirm that the deviations were transient and that the concentration had returned to nominal on the subsequent days.
The test item concentrations were analysed using a HPLC/MS method.

Statistics: Descriptive statistics; one-sided Fisher´s exact test for survival, one-sided Wilcoxon-test for number of eggs/day and fertility and two-sided Dunnett`s test for growth data;

Findings:

Analytical data: Analytical measurements: The control groups were free of test substance contaminations over the whole exposure period.
During the embryo and larval-juvenile exposure phase (until day 60) mean measured concentrations were within the range of ± 20% of the nominal concentrations. Most individually measured concentrations during this exposure phase were within the range ± 20% of the time-weighted mean (TWM) concentrations; however deviations occurred on 2 occasions:
In test group 5 (93.7 µg/L) the measured value in replicate D on day 26 was 138% of TWM. In test group 3 (23.8 µg/L) the measured value in replicate C on day 47 was 161% of TWM. These were the highest measured test concentrations in test group 5 and 3, respectively over the entire experimental period of 257 days. The duration of the high concentration in this test groups was not more than one day.
Both of these deviations were caused by too low fluctuation of the rotameter supplying dilution water to the mixing vessel.

During the juvenile-adult exposure phase (from day 60 to 144) mean measured concentrations of the exposure period were within the range of ± 20% of the nominal concentrations, with the exception of test group 1 (6 µg/L). The measured value in replicate F on day 82 was 58.8 µg/L, approx. a factor of 10 over nominal. This was due to an incorrect stock solution flow rate. On day 83 the concentration dropped to 18.5 µg/L and on day 84 had returned to the acceptable range (6.8 µg/L) As this deviation was extremely high and resulted in a calculated TWM concentration which was not representative of the actual overall exposure concentration, for this test group only, deviations were identified relative to the nominal concentration. Nevertheless, the measured values on days 82-83 were not excluded from the calculation of TWM concentrations. In all test groups the measure values in replicate F on day 96 were 23-36% over the expected nominal concentrations. These values are attributable to a newly prepared stock solution of test substance. The flow rate of pumps delivering stock solution to the mixing tanks was adjusted downwards by 20-30%. Thereafter all measured concentrations were in the required range.

During the reproduction exposure phase (from day 145 until 257) all measured concentrations were within the range ± 20% of the TWM concentrations.

During F1 generation (test days 153 to 215) all measured concentrations were within the range of ± 20% of the TWM concentrations. The measured F1 concentrations were excluded from the calculation of the TWM concentrations because the test solutions originated from the same stock solution as the concurrent F0 generation exposure and since the exposure time overlapped with the F0 reproduction groups, including the F1 concentrations would overweigh days 153 to 215 in the calculation.

The overall time weighted mean (TWM) measured concentrations during the exposure period were within the range of ± 20% of the nominal concentrations. Over the nearly 9 month exposure period, most individually measured concentrations were within the range of ± 20% of the TWM concentrations; however, isolated deviations were recorded in all treatment groups. According to the US EPA OPPTS 850.1000 the goal for limiting variability within a test group over time is maintaining the ration of the highest concentration to the lowest concentration at 1.5:5 or less. If variability exceeds 1.5:1 the exception should be justified. However the most important criterion is the avoidance of overlapping mean test concentrations between test groups. The deviations were transient as confirmed by the subsequent analyses, usually on the following day. At no time did mean measured test concentrations overlap between test levels and in all cases the deviations were concentration increases, consistent with a worst case scenario exposure.

Table 9.2-6: Mean analytically determined concentration (Time weighted mean) for triticonazole during the 257 day FFLC study

mg/L (nominal)	F0-generation day 0-60 embryo-larval-juvenile groups	F0-generation day 60-139 juvenile-adult groups	F0-generation day 145-257 juvenile-adult groups	F1-generation day 153-215
0	Not detectable	Not detectable	Not detectable	Not detectable
0.006	6.4 mg/L (107%) SD: 0.6	9.1 mg/L (152%) SD: 13.3	6.3 mg/L (105%) SD: 0.3	6.4 mg/L (106%) SD: 0.5
0.012	13.3 mg/L (111%) SD: 1.0	13.3 mg/L (111%) SD: 1.4	11.6 mg/L (96%) SD: 0.5	13.3 mg/L (110%) SD: 0.7
0.024	26.1 mg/L (109%) SD: 5.3	24.2 mg/L (101%) 2.9	23.9 mg/L (99%) SD: 1.2	26.1 mg/L (109%) SD: 1.1
0.048	51.2 mg/L (107%) SD: 5.4	47.2 mg/L (98%) SD: 6.5	48.4 mg/L (101%) SD: 3.0	49.8 mg/L (104%) SD: 2.7
0.096	102.4 mg/L (107%) SD: 17	96.2 mg/L (100%) SD: 9.2	93.1 mg/L (97%) SD: 8.6	103.2 mg/L (107%) SD: 7.4

Biological results

There was no adverse treatment-related effect on hatching success, time to hatch, survival, signs of toxicity (appearance/behavior), or reproduction (fertility and fecundity) in the F0-generation fish. Effects on fish length were observed in the F0-generation at the highest test item concentration. The F1-generation fish were exposed from the embryonic stage to approximately 8 weeks post-hatch. There was no adverse treatment-related effect on hatching success, time to hatch, or survival. F1-generation growth was reduced compared to the control at the highest test-item concentration and 14% of fish had morphological deformations after 8 weeks. Based on the design of the fish full life cycle study, a genetic factor (unrelated to test substance) cannot be excluded as the cause of the observed F1-generation responses. The most sensitive effect observed in this study was an adverse effect on growth in the F0 and F1 generations.The results are summarized in Table 9.2.2-4.

Table 9.2-7: Chronic toxicity (FFLC, 257 days) of triticonazole on fathead minnow (*Pimephales promelas*)

Concentration [mg ai/L] nominal			**Control**	**0.006**	**0.012**	**0.024**	**0.048**	**0.096**
Concentration [mg ai/L] Time weighted mean measured over all study parts			**Control**	**0.007**	**0.0125**	**0.0238**	**0.0473**	**0.0937**
Survival	F0	hatching success [%]	92	93	93	93	94	92
		larval survival at end of hatch (day 5) [%]	99	99	99	99	98	99
		juvenile-adult survival (day 60 (after reduction) - day 143) [%]	97	98	97	99	98	96
		juvenile-adult survival (day 60 (after reduction) - day 143) [%]	98	98	100	98	98	98
		adult survival (day 143 (after reduction) -day 257 (sacrifice)) [%]	95	95	90	95	100	95
	F1	hatching success [%]	95	95	94	95	95	95
		larval survival at end of hatch (F1 day 5) [%]	99	100	100	100	99	99
		post hatch survival (F1 day 5 (after reduction) - day F1 day 62 (sacrifice)) [%]	98	100	100	98	100	100
Growth	F0	length on day 31 [cm] photographic measurement (SD)	2.135 (0.0.220)	2.118 (0.176)	2.136 (0.0.156)	2.080 (0.222)	2.122 (0.163)	**2.020** (0.160)**
		deviation from control [%]	--	-0.8	0.0	-2.6	-0.6	-5.4
		length on day 60 [cm] photographic measurement (SD)	3.372 (0.298)	3.494** (0.216)	3.523** (0.252)	3.337 (0.244)	3.399 (0.265)	**3.264* (0.258)**
		deviation from control [%]	--	+3.6	+4.5	-1.0	+0.8	-3.2
		length on day 60 [cm] sacrificed fish (SD)	3.664 (0.187)	3.696 (0.165)	3.670 (0.190)	3.502** (0.218)	3.567 (0.240)	3.604 (0.239)
		deviation from control [%]	--	+0.9	+0.2	-4.4	-2.7	-1.6
		male length on day 143 [cm] sacrificed fish (SD)	5.866 (0.584)	6.367* (0.358)	6.193 (0.482)	6.193 (0.491)	6.071 (0.545)	5.833 (0.458)
		deviation from control [%]	--	+8.2	+5.2	+5.2	+3.1	-0.9
		female length on day 143 [cm] sacrificed fish (SD)	5.433 (0.994)	5.660 (0.974)	5.700 (0.949)	5.133 (0.686)	6.614 (0.318)	5.000 (0.613)
		deviation from control [%]	--	+4.2	+4.9	-5.5	-15.1	-8.0
		male weight on day 143 [g] sacrificed fish (SD)	2.928 (0.718)	3.496 (0.673)	3.205 (0.579)	3.235 (0.577)	3.114 (0.771)	3.046 (0.933)
		deviation from control [%]	--	+19.4	+9.5	+10.5	+6.4	+4.0
		female weight on day 143 [g] sacrificed fish (SD)	2.088 (1.213)	2.252 (1.116)	2.241 (0.932)	1.626 (0.725	1.159 (0.095)	1.625 (0.601)
		deviation from control [%]	--	+7.9	+7.3	-22.1	-44.5	-22.2
		male length on day 257 [cm] sacrificed fish (SD)	6.371 (0.461)	6.025 (0.427)	6.157 (0.326)	6.463 (0.346)	6.325 (0.430)	**5.800* (0.207)**

Concentration [mg ai/L] nominal			**Control**	**0.006**	**0.012**	**0.024**	**0.048**	**0.096**
Concentration [mg ai/L] Time weighted mean measured over all study parts			**Control**	**0.007**	**0.0125**	**0.0238**	**0.0473**	**0.0937**
		deviation from control [%]	--	-5.4	-3.4	+1.4	-0.7	-9.0
		female length on day 257 [cm] sacrificed fish (SD)	5.650 (0.323)	5.818 (0.445)	5.745 (0.169)	5.827 (0.280)	5.467 (0.250)	5.736 (0.344)
		deviation from control [%]	--	+3.0	+1.7	+3.1	-3.2	+1.5
		male weight on day 257 [g] sacrificed fish (SD)	4.114 (1.001)	3.466 (0.718)	3.353 (0.541)	4.343 (0.936)	3.969 (0.882)	3.271 (0.253)
		deviation from control [%]	--	-15.8	-18.5	+5.5	-3.5	-20.5
		female weight on day 257 [g] sacrificed fish (SD)	2.161 (0.401)	2.282 (0.480)	2.181 (0.345)	2.198 (0.365)	1.945 (0.303)	2.255 (0.362)
		deviation from control [%]	--	+5.6	+0.9	+1.7	-10	+4.3
	F1	length on day 186 [cm] photographic measurement (SD)	2.370 (0.247)	2.518** (0.347)	2.306 (0.194)	2.382 (0.169)	2.454 (0.167)	**2.165** (**0.185**)
		deviation from control [%]	--	+6.3	-2.7	+0.5	+3.6	-8.6
		length on day 215 [cm] (SD)	4.341 (0.247)	4282 (0.264)	4.244 (0.252)	4.348 (0.241)	4.268 (0.252)	**3.860** (**0.479**)
		deviation from control [%]	--	-1.4	-2.2	+0.2	-1.7	-11.1
		weight on day 215 [g] (SD)	0.801 (0.170)	0.810 (0.167)	0.772 (0.148)	0.827 (0.155)	0.749 (0.130)	**0.667** (**0.218**)
		deviation from control [%]	--	+1.1	-3.6	-3.3	-6.4	--16.8
Reproduction	F0	Time to maturation/test days when eggs were first observed for each replicate	87/81	91/81	87/93	81/86	82/91	81/81
		sex ratio of fish on day 143 (male/female) [%]	49/51	57/43	50/50	49/51	54/46	45/55
		fertility [%] (mean from the first 6 clutches) (SD)	99.3 (0.6)	99.3 (0.2)	99.4 (0.3)	99.6 (0.5)	99.1 (0.5)	98.2 (0.5)
		eggs/female/day (over the entire spawning period) (SD)	21.1 (3.1)	25.2 (3.4)	19.8 (4.9)	14.1 (5.4)	16.9 (5.5)	16.8 (6.1)
Sublethal effects	F0	Symptoms at day 257	0	0	0	N	0	0
	F1	Symptoms at day 215	0	0	0	0	0	D
Endpoints [mg ai/L]								
Overall NOEC (nominal)			0.048					
Overall NOEC (mean measured)			0.0473					

* Statistically significant differences compared to the control ($p \leq 0.05$)
** Statistically significant differences compared to the control ($p \leq 0.01$)
Deviations which are considered to be substance-related are printed **bold**
SD Standard Deviation
N abdominal extension; D deformations

Conclusion:	The overall NOEC observed in this study is 0.0473 mg ai/ and the respective overall LOEC is 0.0937 mg ai/L mean measured concentration.

Comment RMS:	The study was evaluated following the recommendations of the currently valid test guidelines OPPTS 850.1500, Public draft 1996, OECD 210 (2013) and OECD Detailed Review Paper on Fish Life-Cycle Tests No. 95 **Check of validity criteria:** OPPTS 850.100 (Public draft, 1996) and OECD Detailed Review Paper on Fish Life-Cycle Tests No. 95: No validity criteria stated in these documents OECD 210 (2013): - The dissolved oxygen concentration should be > 60% of the air saturation value throughout the test. The dissolved oxygen concentration was 69-99% of the maximum saturation at test temperature of 25°C. Fulfilled. - The water temperature should not differ by more than ±1.5°C between test chambers or between successive days at any time during the test, and should be within the temperature ranges of 25 ± 1.5°C for *Pimephales promelas.* During the study the temperature was between 24.5 and 26°C. Fulfilled. - The analytical measure of the test concetnrations is compulsory. Fulfilled. - Overall survival of fertilised eggs and post-hatch success in the controls and, where relevant, in the solvent controls should be greater than or equal to 70 and 75%, respectively for *Pimephales promelas*. The hatching success for F0 generation was 92%, for F1 generation 95% and the overall survival for F0 generation was 95% and for F1 generation 98%. Fulfilled. **In addition, the following points deviated from the test guidelines or were not reported in detail:** - Reduction of the group size occurred on day F1 5 to 25 animals per replicate. The possibility of a thinning event is mentioned in OPPTS 850.1400 – Fish Early Life Stage Toxicity Test, but not in OPPTS 850.1500 and OECD 210. **Acceptability of the analytical methods used in the test:** The evaluation of the analytical methods was conducted according to the Guidance Document SANCO/3029/99 rev. 4. Based on the evaluation (see Volume 3 B.5-CA, B.5.1.2.6) it could be demonstrated that the method fulfils the requirements with regard to linearity, specificity, accuracy, repeatability, and limit of quantification. LOQ: 0.001 mg ai/L. **Endpoints:** The RMS agrees on the endpoints given in the study report. ECx-values were not provided with the study report.

	Analysis with Tox RatPro® v.3.2 indicated that no reliable EC_x values could be determined for replicate means of larval length and weight and replicate proportions of hatching success/embryo survival, and fry survival, respectively. NOEC = 0.0473 mg ai/L mean measured concentration LOEC = 0.0937 mg ai/L mean measured concentration **Conclusion of the RMS:** Based on the evaluation of the study the fish full life cycle test is considered valid.

Reference:	**Triticonazole technical: Early life stage toxicity test with Fathead minnow (*Pimephales promelas*)**
Author(s), year:	[redacted] 1998b
Report/Doc. number:	B003479
Guideline(s):	FIFRA Guideline 72-4
GLP:	Yes
Deviations:	Please refer to commenting box below
Validity	Acceptable

Material and methods:

Test substance:	Triticonazole technical (RPA 400727), CAS no.: 131983-72-7, batch no.: OP9750057, purity: 90.52%
Test species:	Fathead minnow (*Pimephales promelas*)
Holding of fish :	No information provided
Number of organisms:	2 replicates per test concentration, control and solvent control, 60 eggs per replicate
Age:	Embryos were ≤ 24 hours old at test initiation.
Type of test:	Flow-through test
Applied concentrations:	
Nominal:	0 (control solvent control), 20, 50, 130, 320 and 790 µg ai/L
Measured (mean):	0 (control, solvent control), 24, 56, 120, 310 and 780 µg ai/L
Solvent:	Acetone
Test conditions:	
Water quality:	Aerated well water, total hardness: 34 to 40 mg/L
Temperature:	25-26 °C
pH:	6.8 – 7.6
O_2 content:	6.1 – 9.5 mg/L (> 60% oxygen saturation)
Conductivity:	150 – 170 µS/cm
Light regime:	Light/dark cycle of 16/8, light intensity approximately 320 to 1100 lux
Methods:	Glass and silicone sealant diluter system and exposure aquaria (39 x 20 x 25 cm),

constant exposure solution volume of approx. 15 L; glass jars (5 cm O.D., 8 cm high) with 40-mesh Nitex® screen bottoms as embryo incubation cups; 6.8 aquarium volumes per 24-hour period, with a 90% replacement time of approx. 8.0 hours.

80 fathead minnow embryos were randomly distributed among two replicates (40 embryos per replicate) of each treatment five days after initiation of the flow-through system. The maximum loading rate at the conclusion of the test was 0.11 g/L.

Test parameters: Dissolved oxygen concentration, pH, temperature, and conductivity were measured daily. Alkalinity and hardness of the control, low and high treatment were analysed weekly.

Analytical measurements: The concentration of triticonazole was analysed in all chambers on day 0, day 5 and weekly thereafter (12, 19, 26, 33, 34).

Statistics: At termination of the early life-stage exposure, data obtained on organism survival at hatch, larval survival and larval growth (total length, wet weight and dry weight) at test termination were analysed to identify significant differences between the treatment group and control organisms. All statistical analyses were conducted at the 95% level of certainty except in the case of Shapiro-Wilks' and Bartlett's Tests, in which the 99% level of certainty was used.

Significant differences in the percent survival were determined after transformation (e.g. arcsine square-root percentage) of the data. Comparisons of larval length measurements between the control and solvent control established that a slight difference exists, therefore, solvent control data was used for statistical comparison to determine treatment level effects for larval length. For the other parameters data from both controls were pooled. As a check on the assumption of homogeneity of variance, implicit in parametric statistics, data for each endpoint were analysed using Bartlett's Test.

In this study, comparisons for embryo survival, larval survival and larval growth were performed using the Williams' test.

The theoretical threshold concentration expected to produce no deleterious effects at the 95% level of certainty was estimated as Maximum Acceptable Toxicant Concentration (MATC) based on the most sensitive of the performance criteria evaluated.

<u>Findings:</u>

Analytical data: The mean measured concentrations of triticonazole ranged between 92 and 120 % of nominal and remained stable throughout the exposure period. No visible sign of undissolved test substance was observed in the mixing chamber, the chemical cells of the diluter system or in any of the exposure solutions.

Biological data:

Table B. 9.2-8: Survival and growth of larvae/fry, 30 day post-hatch

Test concentration [µ ai/L]	Survival at hatch [%]	Survival after 30 d [%]	Length [mm] (SD)	Wet weight [g] (SD)	Dry weight [g] (SD)
Control	96	90	30.7 (1.6)	0.28 (0.04)	0.075 (0.01)
Solvent control	91	93	30.1 (1.6)	0.27 (0.05)	0.071 (0.01)
Pooled control	93	91	nd	0.28 (.0.05)	0.073 (0.01)
24	92	98	29.8 (1.6)	0.26 (0.04)*	0.067 (0.01)*
56	94	98	29.7 (1.7)**	0.25 (0.05)*	0.061 (0.01)*
120	93	98	28.4 (1.8)**	0.22 (0.04)*	0.054 (0.01)*
310	93	96	23.2 (2.5)**	0.12 (0.04)*	0.028 (0.01)*
780	97	36 *	15.1 (3.4)[a]	0.037 (0.02)[a]	0.0078 (0.005)*
LOEC ≤ 24 µg ai/L NOEC < 24 µg ai/L MATC < 24 µg ai/L					

nd…not determined; SD…standard deviation

* Significantly different compared to the pooled control

** Significantly different compared to the solvent control

[a] Growth data for this treatment level was excluded from statistical analysis due to a significant effect on survival.

Conclusion: The overall chronic 30-day-NOEC observed in this study is < 24 µg ai/L and the respective overall chronic 30-day-LOEC is ≤ 24 µg ai/L (based on wet and dry weight of the fry).

Comment RMS: The study was evaluated following the recommendations of the currently valid test guidelines OPPTS 850.1400 (2016) and OECD 210 (2013)

Check of validity criteria:

OPPTS 850.1400 (2016):

- All test vessels and compartments are identical. Fulfilled.
- Treatments are randomly or indiscriminately assigned to individual test vessel locations, individual test organisms were randomly or indiscriminately assigned to test vessels or compartments. Fulfilled.
- A dilution water control (and vehicle (solvent) control, if a vhicle was used) was included in the study. A dilution water control and a solvent control with acetone were tested in the study. Fulfilled.
- The test was begun with embryos at a stage before cleavage of the blastodisc

commences (≤ 24 hour after fertilization). Embryos were ≤ 24 hours old at test initiation. Fulfilled.

- The test was not terminated before the appropriate duration of 32 days (or 28 days of post-hatch) for *Pimephales promelas*. The study lasted until 30 days post-hatch. Fulfilled

- Control survival and hatching success were at least 66% and 70% respectivley for *Pimephales promelas*. Control survival and hatching success were 90% and 96% respectivley. Fulfilled.

- No surfactant or dispersant was used in the preparation of a stock or test solution. (However, adjuvants may be used when testing pesticide typical end-use products). Fulfilled.

OECD 210 (2013):

- The dissolved oxygen concentration should be > 60% of the air saturation value throughout the test. The O_2-content in the study was 6.1 – 9.5 mg/L (83.9 – 95.1% of saturation). Fulfilled.

- The water temperature should not differ by more than ±1.5°C between test chambers or between successive days at any time during the test, and should be within the temperature ranges of 25 ± 1.5°C for *Pimephales promelas.* The temperature during the study was 25-26°C. Fulfilled.

- The analytical measure of the test concentrations is compulsory. During the test, measured concentrations were 92-120% of the nominal concentrations. Fulfilled.

- Overall survival of fertilised eggs and post-hatch success in the controls and, where relevant, in the solvent controls should be greater than or equal to 70 and 75%, respectively for *Pimephales promelas*. In this study spawn used to supply embryos had > 90% fertility/survival. Survival of embryos/fry was 91% in the controls over the study period. Fulfilled

In addition, the following points deviated from the test guidelines or were not reported in detail:

- According to the study report the test aquaria were fabricated of glass and silicone seals. OECD 210 recommends not to using silicone as seal, s it is known to have a strong capacity to absorb lipophilic substances.

- No details on the holding of the brood stock were provided.

Acceptability of the analytical methods used in the test: The evaluation of the analytical methods was conducted according to the Guidance Document SANCO/3029/99 rev. 4. Based on the evaluation (see Volume 3 B.5-CA, B.5.1.2.6) the analytical method is considered valid to quantify the amount of triticonazole in fresh and seawater.

LOQ: 0.0225 mg/L

Endpoints:

The RMS agrees on the endpoints given in the study report.

EC_x-values were not provided with the study report. The RMS estimated EC_x-values using Tox RatPro® v.3.2

NOEC < 24 µg ai/L nominal concentration

LOEC ≤ 24 µg ai/L nominal concentration

MATC < 24 µg ai/L nominal concentration

$EC_{10\ length}$ = 155.63 µg ai/L (95% C.I. = 133.24 – 176.99 µg ai/L) nominal concentration

reliability based on normalised width of C.I. = excellent

$EC_{20\ length}$ = 281.71 µg ai/L (95% C.I. = 253.44 – 739.68 µg ai/L) nominal concentration

$EC_{50\ length}$ = 776.95 µg ai/L (95% C.I. = 739.68 – 815.10 µg ai/L) nominal concentration

$EC_{10\ wet\ weight}$ = could not be calculated

$EC_{20\ wet\ weight}$ = could not be calculated

$EC_{50\ wet\ weight}$ = could not be calculated

$EC_{10dry\ weight}$ = 36.58 µg ai/L (95% C.I. = 26.64 – 46.81 µg ai/L) nominal concentration

reliability based on normalised width of C.I. = fair

$EC_{20dry\ weight}$ = 77.25 µg ai/L (95% C.I. = 61.76 – 92.22 µg ai/L) nominal concentration

$EC_{50dry\ weight}$ = 238.86 µg ai/L (95% C.I. = 214.76 – 263.66 µg ai/L) nominal concentration

Conclusion of the RMS: Based on the evaluation of the study the fish early life stage test is considered valid.

Reference:	**Triticonazole technical: Early life stage toxicity test with Fathead minnow (*Pimephales promelas*)**
Author(s), year:	[redacted] 1998c
Report/Doc. number:	Document no.: C044319
Guideline(s):	FIFRA Guideline 72-4; OPPTS Draft Guideline 850.1400
GLP:	Yes
Deviations:	Please refer to commenting box below
Validity	Acceptable

Material and methods:

Test substance:	Triticonazole technical (RPA 400727), batch no.: OP9750057, purity: 90.52%
Test species:	Fathead minnow (*Pimephales promelas*)
Holding of fish :	No information provided
Number of organisms:	2 replicates per test concentration, control and solvent control, 60 eggs per replicate
Age:	Embryos were ≤ 24 hours old at test initiation.
Type of test:	Flow-through test
Applied concentrations:	
Nominal:	0 (control solvent control), 1.4, 3.6, 9.0, 22 and 56 µg ai/L
Measured (mean):	0 (control, solvent control), 1.7, 3.7, 8.7, 21 and 51 µg ai/L
Solvent:	Acetone
Test conditions:	
Water quality:	Aerated well water, total hardness: 36 to 40 mg/L
Temperature:	25 - 26 °C
pH:	6.7 – 7.5
O_2 content:	5.2 – 8.7 mg/L (> 60% oxygen saturation)
Conductivity:	160 – 170 µS/cm
Light regime:	Light/dark cycle of 16/8, light intensity approximately 220 to 1100 lux
Methods:	Glass and silicone sealant diluter system and exposure aquaria (39 x 20 x 25 cm), constant exposure solution volume of approx. 15 L; glass jars (5 cm O.D., 8 cm high) with 40-mesh Nitex® screen bottoms as embryo incubation cups; 6.9 aquarium volumes per 24-hour period, with a 90% replacement time of approx. 7.0 hours. 80 fathead minnow embryos were randomly distributed among two replicates (40 embryos per replicate) of each treatment five days after initiation of the flow-through system. The maximum loading rate at the conclusion of the test was 0.13 g/L.
Test parameters:	Dissolved oxygen concentration, pH, temperature, and conductivity were measured daily. Alkalinity and hardness of the control, low and high treatment

were analysed weekly.

Analytical measurements: The concentration of triticonazole was analysed in all chambers on day 0, day 4 and weekly thereafter (11, 18, 25, 32, 34). In addition the lowest treatment level was resampled and analysed on day 12.

Statistics: At termination of the early life-stage exposure, data obtained on organism survival at hatch, larval survival and larval growth (total length, wet weight and dry weight) at test termination were analysed to identify significant differences between the treatment group and control organisms. All statistical analyses were conducted at the 95% level of certainty except in the case of Shapiro-Wilks' and Bartlett's Tests, in which the 99% level of certainty was used.

Significant differences in the percent survival were determined after transformation (e.g. arcsine square-root percentage) of the data. Since during this study comparisons indicated that the presence of acetone in the two control solutions did not affect survival of organisms at hatch, larval survival or larval growth of the test organisms, the data for the control and the solvent control were pooled.

As a check on the assumption of homogeneity of variance, implicit in parametric statistics, data for each endpoint were analysed using Bartlett's Test.

In this study, comparisons for embryo survival, larval survival and larval growth were performed using the Williams' test.

The theoretical threshold concentration expected to produce no deleterious effects at the 95% level of certainty was estimated as Maximum Acceptable Toxicant Concentration (MATC) based on the most sensitive of the performance criteria evaluated.

Findings:

Analytical data: The mean measured concentrations of triticonazole ranged between 92 and 120 % of nominal and remained stable throughout the exposure period. No visible sign of undissolved test substance was observed in the mixing chamber, the chemical cells of the diluter system or in any of the exposure solutions.

Biological data:

Table 9.2-9: Survival and growth of larvae/fry, 30 day post-hatch

Test concentration [µ ai/L]	Survival at hatch [%]	Survival after 30 d [%]	Length [mm] (SD)	Wet weight [g] (SD)	Dry weight [g] (SD)
Control	88	90	31.6 (2.1)	0.29 (0.05)	0.076 (0.02)
Solvent control	86	88	32.2 (1.8)	0.31 (0.06)	0.083 (0.02)
Pooled control	87	89	31.9 (2.0)	0.30 (.0.05)	0.079 (0.02)
1.7	87	94	32.5 (2.0)	0.32 (0.06)	0.085 (0.02)
3.7	84	91	32.0 (2.0)	0.31 (0.06)	0.083 (0.02)

Test concentration [µ ai/L]	Survival at hatch [%]	Survival after 30 d [%]	Length [mm] (SD)	Wet weight [g] (SD)	Dry weight [g] (SD)
8.7	86	91	31.7 (1.9)	0.31 (0.07)	0.079 (0.02)
21	86	98	31.5 (2.3)	0.29 (0.06)	0.076 (0.02)
51	88	90	30.5 (2.0)*	0.27 (0.06)*	0.067 (0.02)*
LOEC = 51 µg ai/L NOEC = 21 µg ai/L MATC > 21 < 51 µg ai/L					

nd…not determined; SD…standard deviation
* Significantly different compared to the pooled control

Conclusion: The overall chronic 30-day-NOEC observed in this study is 21 µg ai/L and the respective overall chronic 30-day-LOEC is 51 µg ai/L. The MATC is > 21 < 51 µg ai/L.

Comment RMS: The study was evaluated following the recommendations of the currently valid test guidelines OPPTS 850.1400 (2016) and OECD 210 (2013)

Check of validity criteria:

OPPTS 850.1400 (2016):

- All test vessels and compartments are identical. Fulfilled.
- Treatments are randomly or indiscriminately assigned to individual tes vessel locations, individual test organisms were randomly or indiscriminately assigned to test vessels or compartments. Fulfilled.
- A dilution water control (and vehicle (solvent) control, if a vhicle was used) was included in the test. A dilution water control and a solvent control with acetone were tested in the study. Fulfilled.
- The test was begun with embryos at a stage before cleavage of the blastodisc commences (≤ 24 hour after fertilization). Embryos were ≤ 24 hours old at test initiation. Fulfilled.
- The test was not terminated before the appropriate duration of 32 days (or 28 days of post-hatch) for *Pimephales promelas*. Fulfilled.
- Control survival and hatching success were at least 66% and 70% respectivley for *Pimephales promelas*. Control survival and hatching success were 90% and 88% respectivley. Fulfilled.
- No surfactant or dispersant was used in the preparation of a stock or test solution. (However, adjuvants may be used when testing pesticide typical end-use products). Fulfilled.

OECD 210 (2013):

- The dissolved oxygen concentration should be > 60% of the air saturation value

	throughout the test. The O2-content in the study was 6.7 – 9.5 mg/L (82.6 – 93.9% of saturation). Fulfilled. - The water temperature should not differ by more than ±1.5°C between test chambers or between successive days at any time during the test, and should be within the temperature ranges of 25 ± 1.5°C for *Pimephales promelas.* The temperature during the study was 25-26°C. Fulfilled. - The analytical measure of the test concetnrations is compulsory. During the test, measured concentrations were 92-120% of the nominal concentrations. Fulfilled. - Overall survival of fertilised eggs and post-hatch success in the controls and, where relevant, in the solvent controls should be greater than or equal to 70 and 75%, respectively for *Pimephales promelas*. In this study spawn used to supply embryos had > 80% fertility/survival. Survival of embryos/fry was 89% in the controls over the study period. Fulfilled. **In addition, the following points deviated from the test guidelines or were not reported in detail:** - According to the study report the test aquaria were fabricated of glass and silicone seals. OECD 210 recommends not to using silicone as seal, s it is known to have a strong capacity to absorb lipophilic substances. - No details on the holding of the brood stock were provided. **Acceptability of the analytical methods used in the test:** The evaluation of the analytical methods was conducted according to the Guidance Document SANCO/3029/99 rev. 4. Based on the evaluation (see Volume 3 B.5-CA, B.5.1.2.6) the analytical method is considered valid to quantify the amount of triticonazole in fresh and seawater. LOQ: 0.45 µg/L **Endpoints:** The RMS agrees on the endpoints given in the study report. Analysis with Tox RatPro® v.3.2 showed that no reliable EC_x values could be determined LOEC = 51 µg ai/L nominal concentration NOEC = 21 µg ai/L nominal concentration MATC > 21 < 51 µg ai/L nominal concentration **Conclusion of the RMS:** Based on the evaluation of the study the fish early life stage test is considered valid.

Reference:	**BASF 595F - Early life stage toxicity test with sheepshead minnow (*Cyprinodon variegatus*)**
Author(s), year:	[redacted] 2006a
Report/Doc. number:	BASF Reg. Doc. No.: 2006/7007245
Guideline(s):	OPPTS Draft Guideline 850.1400
GLP:	Yes
Deviations:	Please refer to the commenting box below
Validity	Acceptable

Material and methods:

Test substance:	BAS 595 F (Triticonazole), CAS no. : 131983-72-7, Reg. No.: 4378513; purity : 90.3%
Test species:	Sheepshead Minnow (*Cyprinodon variegatus*)
Holding of fish :	No information provided
Number of organisms:	4 replicates per test concentration, control and solvent control, 40 eggs per replicate
Age:	Embryos were ≤ 30 hours old at test initiation.
Type of test:	Flow-through test
Applied concentrations:	
Preliminary test 1:	0.5, 1.0, 2.0, 4.0, 8.0 mg ai/L (nominal)
Preliminary test 2:	6.3, 13, 25, 50, 100 µg ai/L (nominal)
Nominal:	0 (control solvent control), 7.5, 15, 30, 60 and 120 µg ai/L
Measured (mean):	0 (control, solvent control), 8.3, 16, 33, 64 and 120 µg ai/L
Solvent:	Dimethylformamide (DMF)
Test conditions:	
Water quality:	Natural filtered seawater recirculated within an epoxy-coated concrete holding reservoir. The dilution water was prepared by adjusting the salinity to 20 ± 4 ‰ with laboratory well water. The seawater used for this study had a salinity of 19 to 21 ‰
Temperature:	24-26 °C
pH:	7.6 – 8.1
O_2 content:	3.1 – 8.0 mg/L; The dissolved oxygen concentration dropped below 60% saturation in the treatment concentrations 12, 30, and 120 and the solvent control for < 24 hours. Gentle, oil-free aeration was initiated on test day 29.
Salinity:	19-21 ‰
Light regime:	Light/dark cycle of 16/8, light intensity 570 to 1000 lux
Methods:	Glass and silicone sealant diluter system and exposure aquaria (30 x 14.5 x 20 cm), constant exposure solution volume of approx. 6.5 L; glass jars (5 cm O.D., 8 cm high) with 475-µm nylon screen bottoms as embryo incubation cups; 7.5

aquarium volumes per 24-hour period, with a 90% replacement time of approx. 7.0 hours.

80 sheepshead minnow embryos were randomly distributed among four replicates (20 embryos per replicate) of each treatment on test day 6. The maximum loading rate at the conclusion of the test was 0.017 g/L.

Test parameters: Dissolved oxygen concentration, pH, salinity and temperature were measured at test initiation and weekly thereafter until test termination. In addition temperature, pH, salinity and dissolved oxygen were measured in alternating replicates of one test vessel of each concentration and the controls, daily. Furthermore temperature was continuously monitored in one control replicate vessel.

Analytical measurements: The concentration of triticonazole was analysed in all chambers on day 0, day 6, 12, 19, 26, and 33.

Statistics: At termination of the early life-stage exposure, data obtained on organism survival at hatch, larval survival and larval growth (total length, wet weight and dry weight) at test termination were analysed to identify significant differences between the treatment group and control organisms. All statistical analyses were conducted at the 95% level of certainty except in the case of Shapiro-Wilks' and Bartlett's Tests, in which the 99% level of certainty was used.

For this study, analyses of survival, length and weight using the Student's t-Test established no significant difference between the dilution water control and solvent control. Therefore, statistical comparisons to determine treatment effects for survival, length and weight were performed utilizing the pooled control data.

As a check on the assumption of homogeneity of variance, implicit in parametric statistics, data for each endpoint were analysed using Bartlett's Test.

All endpoints met the assumptions for normality and homogeneity of variance (Sokal and Rohlf, 1981). Therefore, the performance of organisms exposed to eacht treatment level of the test substance was compared with the performance of the pooled control using Williams' Test.

TOXSTAT® version 3.5 was used to perform the statistical computation. The theoretical threshold concentration expected to produce no deleterious effects at the 95% level of certainty was estimated as Maximum Acceptable Toxicant Concentration (MATC) based on the most sensitive of the performance criteria evaluated.

Findings:

Analytical data: The mean measured concentrations of triticonazole ranged between 100 and 110 % of nominal and remained stable throughout the exposure period. Throughout the study period, all exposure solutions were observed to be clear and colourless.

Table 9.2-10: Survival and growth of larvae/fry, 28 day post-hatch

Test concentration [µ ai/L]	Survival at hatch [%]	Survival after 28 d [%]	Length [mm] (SD)	Dry weight [g] (SD)
Control	98	96	22.2 (1.7)	0.0402 (0.0084)
Solvent control	96	96	22.7 (0.85)	0.0428 (0.0068)
Pooled control	97	96	22.5 (1.4)	0.0415 (0.0077)
8.3	97	95	22.3 (1.4)	0.0410 (0.0081)
16	100	99	22.0 (1.8)	0.0386 (0.0097)
33	96	93	22.1 (1.6)	0.0398 (0.0083)
64	97	90	22.1 (1.9)	0.0402 (0.0099)
120	99	94	22.0 (1.3)	0.0396 (0.0079)
LOEC >120 µg ai/L NOEC = 120 µg ai/L MATC > 120 µg ai/L				

SD…standard deviation
* Significantly different compared to the pooled control
** Significantly different compared to the solvent control

Conclusion: The overall chronic 28-day-NOEC observed in this study is 120 µg ai/L and the respective overall chronic 28-day-LOEC is > 120 µg ai/L. The MATC is > 120 µg ai/L.

Comment RMS: The study was evaluated following the recommendations of the currently valid test guidelines OPPTS 850.1400 (2016) and OECD 210 (2013)

Check of validity criteria:

OPPTS 850.1400 (2016):

- All test vessels and compartments are identical. Fulfilled.
- Treatments are randomly or indiscriminately assigned to individual test vessel locations, individual test organisms were randomly or indiscriminately assigned to test vessels or compartments. Fulfilled.
- A dilution water control (and vehicle (solvent) control, if a vhicle was used, was included in the test. A dilution water control and a solvent control with dimethylformamid were tested in the study. Fulfilled.
- The test was begun with embryos at a stage before cleavage of the blastodisc commences (≤ 24 hour after fertilization). Embryos were < 30 hours old at test initiation. Fulfilled.
- The test was not terminated before the appropriate duration of 32 days (or 28 days of post-hatch) for *Cyprinodon variegatus*. The study lasted for 28 days post-hatch. Fulfilled.
- Control survival and hatching success were at least 75% and 80% respectivley for *Cyprinodon variegatus*. Control survival and hatching success were 98% and

96% respectivley. Fulfilled.
- No surfactant or dispersant was used in the preparation of a stock or test solution. (However, adjuvants may be used when testing pesticide typical end-use products). Fulfilled.

OECD 210 (2013):
- The dissolved oxygen concentration should be > 60% of the air saturation value throughout the test. The O_2-content in the study was 3.1 – 8.0 mg/L (37.6 for < 24 hours – 100.1% of saturation).
- The water temperature should not differ by more than ±1.5°C between test chambers or between successive days at any time during the test, and should be within the temperature ranges of 25 ± 1.5°C for *Cyprinodon variegatus*. The temperature during the study was 24-26°C. Fulfilled.
- The analytical measure of the test concentration is compulsory. During the test, measured concentrations were 100 - 110% of the nominal concentrations. Fulfilled.
- Overall survival of fertilised eggs and post-hatch success in the controls and, where relevant, in the solvent controls should be greater than or equal to 75 and 80%, respectively for *Cyprinodon variegatus*. In this study spawn used to supply embryos had 89% fertility/survival. Survival of embryos/fry was 89% in the control and solvent control (pooled values) over the study period. Fulfilled.

In addition, the following points deviated from the test guidelines or were not reported in detail:
- According to the study report the test aquaria were fabricated of glass and silicone seals. OECD 210 recommends not to using silicone as seal, s it is known to have a strong capacity to absorb lipophilic substances.
- No details on the holding of the brood stock were provided.

Acceptability of the analytical methods used in the test: The evaluation of the analytical methods was conducted according to the Guidance Document SANCO/3029/99 rev. 4. Based on the evaluation (see Volume 3 B.5-CA, B.5.1.2.6) the analytical method is considered valid to quantify the amount of triticonazole in fresh and seawater.

LOQ: 0.45 µg/L

Endpoints:

The RMS agrees on the endpoints given in the study report.

EC_x-values were estimated by Probit analysis using linear max. likelihood regression. The observations used were replicate means (length and weight) or replicate proportions (hatching success/embryo survival, fry survival).

LOEC > 120 µg ai/L mean measured concentration

	NOEC = 120 µg ai/L mean measured concentration MATC > 120 µg ai/L mean measured concentration Analysis with Tox RatPro® v.3.2 showed that no reliable EC_x values could be determined **Conclusion of the RMS:** Based on the evaluation of the study the fish early life stage test is considered valid.

In addition to the fish early life stage tests and the fish full life cycle tests a fish juvenile growth test (28 days) with the rainbow trout was submitted. According to the new data requirements fish juvenile growth tests are no longer a data requirement. Hence, the study was not evaluated in detail for the renewal of the EU approval. The study summary given in the DAR for the first EU approval is presented below as additional information.

[redacted] ***1996a – Triticonazole: fish, juvenile grwoth test – 28 days***

Document number: R013166

Material and methods:

The effect of triticonazole on growth of rainbow trout (Oncorhynchus mykiss) was assessed in a 28 d test under flow through conditions. At test initiation fish had a mean length of 6 cm (s.d. = 0.1) and a mean weight of 3.38 g (s.d. = 0.26). 16 individuals per concentration, control and solvent control were used for testing. Based on results of preliminary range finding studies the nominal test concentrations were as follows: 0.01, 0.032, 0.1, 0.32 and 1 mg/l. Tween 80-dimethylformamide was used as a solvent. Test conditions: Temperature, 14 °C; pH, 7.4 – 7.6, dissolved O2, 8.1 – 10 mg/l; total hardness as CaCO3, 104 – 111 mg/l. Temperature, pH, dissolved O2 and concentrations of test substance were recorded daily throughout the study. Fish were weighted on days 0, 14 and 28.

Findings:

Except on day 22 (nominal concentration of 0.032 mg/l) all measured concentrations were above 80 % of nominal concentrations, therefore results were based on nominal concentrations. No dead fish were observed at any test concentration and controls. The mean weight of fish in control and solvent control was 3.46 and 3.45 on day 0. On day 28 the mean weight of fish in control and solvent control was 8.61 and 8.64. Inhibition of growth was observed at the test concentrations of 0.032, 0.1, 0.32 and 1 mg/l. No effect was observed at the concentration of 0.01 mg/l.

Assessment: 28 d EC20 = 0.31 mg/l, 28 d NOEC = 0.01 mg/l

B.9.2.3. Potential for endocrine disruption

The applicant provided following statement (in *italic*), For summary and discussion of the study please refer to Volume 3 Part 6-CA.:

Based on the physical, chemical and structural characteristics of the active substance triticonazole as well as based on results of available long-term fish studies (see summaries for ELS and FLC studies above) and studies on terrestrial vertebrates (see chapter MCA-8.1.5) there is no indication of endocrine disrupting properties of this active substance.

This is supported by a peer-reviewed scientific study by Hermsen et al. (2011) on relative embryotoxicity of two classes of chemicals in a modified zebrafish embryotoxicity test. Triticonazole showed minor effects only in the highest concentration tested and was the least toxic of all the triazoles tested. Thus, this paper raised no concerns with regards to endocrine disrupting (ED) effects. The literature study was considered as relevant but not reliable (RI 3); thus, reference is made to this study but no study summary is provided. For details please see the literature search and evaluation files also provided within the submission for Annex I Renewal.

Finally, no endocrine activity of triticonazole was detected by several toxicological impact assessments, i.e. triticonazole did not exert significant androgenic or anti-androgenic effects and no estrogenic or anti-estrogenic effects (see MCA 5.8.3).

Thus, based on available information it can be concluded that there is no ecotoxicological concern regarding the ED potential of triticonazole and no further studies are required.

Comment RMS: As at the time of evaluation no guidance was available, a conclusion on the potential of endocrine disruption is not possible.

B.9.2.4. Bioconcentration in fish

As the log P_{OW} of the active substance and its metabolite RPA 406203 (Reg.No. 5079359) is 3.3 and 3.5, respectively, evaluation of the bioconcentration potential in fish is needed. No new bioconcentration studies have been provided. Therefore the old bioconcentration studies were re-evaluated and are summarized below**.** No fish bioaccumulation study is available for RPA 406203. RPA 406203 is the Z-isomer of the parent, as a likely assumption therefore the BCF fish of the parent was used for the risk assessment. For further details please refer to Volume 3 B9-CP.

Reference:	**A fish bioaccumulation and depuration study on RPA 400727**
Author(s), year:	[redacted]., 1994a
Report/Doc. number:	R013103
Guideline(s):	US EPA guideline 165-4 (1982); OECD-305E
GLP:	Yes
Deviations:	Please refer to the commenting box below
Validity:	Not acceptable

Materials and Methods:

Test material	[^{14}C]-RPA 400727
Lot/Batch #	0991
Specific activity	250 µCi mL^{-1} (32 mCi/mMol)
Radiochemical purity	> 99%
Water	Sand filtered lake water
Carrier solvent	1% acetone

Test organism:	*Oncorhynchus mykiss*
Size	Not reported
Body weight	Not reported
Loading	12 mg/L/day (approx. values based on weight of fish at sampling)
Source	[redacted]
Diet/Food	Fish were fed 5 days a week BP Mainstream fry 02, BP mainstream Nutrition, UK Limited, Wincham, Northwich, Cheshire during holding and test periods. Any excess food was removed after 20 min.
Acclimatisation period:	5 days prior to test initiation, with increased temperature from 10 t0 15 °C; following temperature acclimation fish used in Phase I were held under the experimental conditions for a further 21 days, fish used in Phase II were held for only 10 days

Environmental conditions	
Temperature	15 ± 1°C
Photoperiod	12 hours light : 12 hours dark
pH	6.8 (measured fortnightly)
Dissolved oxygen	Not measured
Total hardness	Not measured
Alkalinity	21.7 mg/L carbonate (measured fortnightly)
Conductivity	Not measured

Study design:

Experimental conditions:

The bioaccumulation of [^{14}C]-RPA 400727 (purity > 99%) in rainbow trout (*Oncorhynchus mykiss*) was investigated in a flow-through system at nominal exposure concentrations of 0.1 and 0.4 mg/L.

As a consequence of technical difficulties part I of the study was performed in two phases.

Phase I: accumulation and depuration, which examined accumulation and initial depuration.

Phase II: depuration, which examined depuration only.

Identification of metabolites isolated from fish sampled during the course of this study was conducted in a different laboratory and is presented in a separate part of the study report.

The test was conducted in 20 L enclosed circular glass flasks. Two control flasks, two 0.1 mg/L and two 0.4 mg/L flasks were used for the accumulation and initial depuration phase each containing 38 fish. One flask for

control and one for the treatment concentrations was used each with 35 fish per tank for the depuration phase only. The flow through rate was 100 mL/min, which equated to 7.2 volume additions over 24 hours.
The number, quantitiy and identity of radiolabelled degradates in edible tissue and viscera from fish killed on at least two time points during the accumulation phase and on day 14 of the depuration phase was determined.

Observations:
Fish were observed daily, regarding morbidity and mortality, at least 5 times a week, with additional observations made throughout the working day as necessary. Body weights and fork lengths were recorded at sampling only. Temperature was recorded for the individual flasks throughout the experiment.
Five test and 5 control fish were taken on days 0, 1, 3, 7, 10, 14 (only 2 for 0.4 mg/L), 21 (only 2 for 0.4 mg/L), 28 and 42 (0 for 0.4 mg/L) of the accumulation and initial depuration phase and 4 fish were taken on days 28, 29, 31, 35, 38 and 42 of the depuration phase. After killing, fish were weighed and their fork length recorded. Each fish was dissected into edible tissue (muscle fillet) and non-edible (viscera and residual carcass) fractions and assayed for radioactivity.
After completing the in-life phase of the study, fish were despatched, transferred to the other laboratory and stored at approximately – 20°C prior to analysis. The samples were thawed and dissected into edible and non-edible fractions. These samples were taken for enzyme deconjugation, HPLC and LC-MS analysis.
The pooled extracts from the edible and non-edible fraction of rainbow trout were investigated using LC-MS to provide structural elucidation of the major metabolites present.

Calculations:
Bioconcentration factors, for test fish tissues and whole fish were calculated from steady state concentration of the test article in fish.
Uptake rate and depuration rate constants k_1 and k_2
Identification of radiolabelled components in the exposure water
Isolation/identification of metabolites in edible and non-edible fractions from rainbow trout
Structural elucidation of radiolabelled metabolites by mass spectrometry

Results and discussion:

Exposure concentration:
At the lower concentration (0.1 mg/l) aqueous levels were 90% (0.09 ± 0.003 mg/L) and 72% (0.072 ± 0.003 mg/L) of the nominal concentration for phase 1 and 2 respectively. At the higher concentration (0.4 mg/L) aqueous levels were 40% (0.16 ± 0.01 mg/L) and 45% (0.18 ± 0.02 mg/L) of the nominal concentration for phase 1 and 2 respectively.

Residue in fish:
The rate of uptake, expressed as k1, in either edible or non-edible tissues were independent of exposure concentration. It appears to be greater in the non-edible fraction than in the edible fraction.
At 0.1 mg/L, steady state was achieved within 24 hours with 0.51 and 1.87 µg equivalents of [^{14}C]-RPA 400727

g/wet tissue in edible and non-edible fractions respectively. At 0.4 mg/L steady state concentrations were again achieved well within the first sample point (24 hours) providing steady state concentrations in edible and non-edible fractions of 1.14 and 3.45 µg equivalents of [^{14}C] RPA 400727.
Bioconcentration factors were low in all fractions sampled and were found to be independent of exposure level. The BCF for the whole fish were 11.67 and 12.75; in the non-edible fraction 20.78 and 21.56 and in the edible fraction 5.67 and 7.12 at 0.1 and 0.4 mg/L nominal exposure concentrations respectively.

Elimination of radioactivity from the fish was rapid. Concentrations of [^{14}C]-RPA 400727 equivalents in edible fraction were below the limit of detection within the first post exposure sample interval. Conversely the elimination of radioactivity from non-edible fraction was considerably slower with levels only falling below the limit of detection between 3 to 7 days post-exposure. Rates of elimination of radioactivity for edible fraction were not dependent on the exposure level. For non-edible fractions at the high exposure concentration the rate of elimination appeared to be slower.

The radioactivity in edible and non-edible fractions was below the limit of detection after 14 d of depuration. Therefore, identification and quantification of metabolites was conducted only for samples taken at days 21 and 28 of uptake phase.
Tissue extracts from test fish sampled on day 21 and 28 were examined by HPLC and LC-MS analysis. Principal radioactive components were, RPA 404886, RPA 406972 and RPA 406341.

Table 9.2-11: Bioconcentration factors, uptake and depuration constants of radioactivity in all fractions of rainbow trout

Parameter	Tissue Fraction		
	Edible	Non-edible	Whole Fish
0.1 mg/L			
Uptake rate constant k1, h^{-1}	0.020	0.085	n.d.
Depuration rate constant, k2, h^{-1}	0.17	0.042	n.d.
Bioconcentration Factor	5.67	20.78	11.67
0.4 mg/L			
Uptake rate constant k1, h^{-1}	0.021	0.160	n.d.
Depuration rate constant, k2, h^{-1}	0.077	0.022	n.d.
Bioconcentration Factor	7.12	21.56	12.75

n.d…not determined

Mortality was 0.9% and 3% prior to the accumulation and depuration phases, respectively. No overt pharmacological or toxicological signs were observed in the test animals which could have been attributed to the test article. Mortalities were not test article related but due to asphyxiation resulting from a blockage of the inlet tubing.

Conclusion:

The bioaccumulation of [^{14}C]-triticonazole (purity > 99%) in rainbow trout (*Oncorhynchus mykiss*) was investigated in a flow-through system at a nominal exposure concentrations of 0.1 mg/L and 0.4 mg/L. In whole fish, apparent steady-state was achieved after 24 h exposure to the test material.

Concentrations of [14C]-RPA 400727 equivalents in edible fraction were below the limit of detection within the first post exposure sample interval. Conversely the elimination of radioactivity from non-edible fraction was considerably slower with levels only falling below the limit of detection between 3 to 7 days post-exposure.

Comment RMS:	The study was evaluated following the recommendations of the currently valid test guideline OECD 305 (2012). **Check of validity criteria:** - The water temperature variation is less than ± 2 °C, because large deviations can affect biological parameters relevant for uptake and depuration as well as cause stress to animals. In the current study the temperature was 15 ± 1 °C. Fulfilled. - The concentration of dissolved oxygen did not fall below 60% saturation. In the current study the oxygen concentration was not measured. Not fulfilled. - The concentration of the substance in the chambers is maintained within ± 20% of the mean of the measured values during the uptake phase. In the current study the concentration was only 40% of the nominal concentration at the higher treatment level. Not fulfilled. - The concentration of the test substance was below its limit of solubility in water. Fulfilled. - The mortality or other adverse effects/disease in both control and treated fish is less than 10% at the end of the test; where the test is extended over several weeks or months, death or other adverse effects in both sets of fish should be less than 5% per month and not exceed 30% in all. Significant differences in average growth between the test and the control groups of sampled fish could be an indication of a toxic effect of the test chemical. In the current study the mortality was 0.9% and 3% prior to the accumulation and depuration phases, respectively. Fulfilled. Mortality in control is not stated. **In addition, the following points deviated from the test guidelines or were not reported in detail:** - According to OECD 305, during the test, dissolved oxygen, TOC, pH and temperature should be measured in all test and control vessels. In the current study TOC and dissolved oxygen were not measured. - According to OECD 305, the lipid content should be determined on the same biological material as is used to determine the concentration of the test substance at least at the start and end of the uptake phase and at the end of the depuration phase. In the current test the lipid content was not estimated. If degradates representing ≥ 10% of total radiolabelled residues in the fish tissue

	are identified and quantified, then it is also recommended to identify and quantify degradates in the test water. Should this not be feasible, this should be explained in the report. - According to OECD 305, at the start of the test, five to ten fish from the stock population need to be weighed individually and their total length measured. In the current study it is not reported that fish weight and length of fish were estimated at the start of the test. Also there are no results given of the weighing and measurement during sampling occasions. **Endpoints:** The study report in general is a rather confusing, some informations are missing or are reported in an unclear way. The endpoints derived from this study are: BCF_{SS}edible: 7.12 BCF_{SS}non-edible: 21.56 BCF_{SS}whole fish: 12.75 The values cannot be verified as the fish weight was not reported. Furthermore O_2 saturation and TOC which may influence bioavailability were not measured. **Conclusion of the RMS:** Based on the evaluation of the study the fish bioaccumulation test is considered not valid. Essential information is missing in the study report (see above). Additionally, the measured concentration deviates for more than 20% from the nominal concentration, which is a validity criterion. Therefore it is not considered to be acceptable for use in the risk assessment

Reference:	**[^{14}C]-Triticonazole bioaccumulation test in bluegill sunfish**
Author(s), year:	[redacted] 1996a
Report/Doc. number:	C017724
Guideline(s):	US EPA guideline 165-4
GLP:	Yes
Deviations:	Please refer to the commenting box below
Validity:	Questionable

Materials and Methods:

Test material	[^{14}C]-Triticonazole
Lot/Batch #	1191 1194
Specific activity	9.9 µCi mg^{-1}
Radiochemical purity	> 97%
Water	Charcoal-filtered dechlorinated tap water
Solvent	N, N-dimethylformamide (DMF)

Test organism:	*Lepomis macrochirus*
Size	27– 50 mm
Body weight	0.504 – 2.826 g
Loading	Not reported
Source	[redacted]
Diet/Food	Fish were fed daily with Salmon Fry Diet, Crumb 01, BP Nutrition, during holding and test periods. The diet contained 50% protein, 20% oil, 12.5% carbohydrate, 21.85 mJ/kg Gross Energy and 19.35 mJ/kg Digestable Energy. During the test, any excess food was removed after 30 min. In nine occasions, excess food was removed at other intervals after feeding but always within a relatively short period (≤ 6 h).
Acclimatisation period:	5 weeks prior to test initiation

Environmental conditions	
Temperature	20.4 – 23.0°C
Photoperiod	16 hours light: 8 hours dark
pH	7.2 – 8.2
Dissolved oxygen	64 – 96%
Total hardness	48 – 80 mg $CaCO_3$/L
Alkalinity	38 – 46 mg $CaCO_3$/L
Conductivity	160 – 210 µS/cm

Study design :

Experimental conditions:

The bioaccumulation of [^{14}C]-Triticonazole (purity > 97%) in bluegill sunfish (*Lepomis machrochirus*) was investigated in a flow-through system at nominal exposure concentrations of 89 µg/L. Following pre-equilibration of the test system for 3 days, fish were exposed to triticonazole for 28 days followed by a 14 days depuration period. The test was conducted in two aerated 60 L glass tanks with 161 fish receiving triticonazole stock solution and charcoal-filtered dechlorinated tap water (300 L/day) and 149 control fish receiving dilution water alone. The fish were fed daily during the study.

Observations:

Fish were observed daily for signs of disease, stress, irritation and other effects.

The pH, temperature range, conductivity and dissolved oxygen concentration were measured daily in each tank. Total hardness was measured at weekly intervals.

Water samples were taken daily from each tank throughout the pre-equilibration and uptake phases and on Days 3, 7 and 14 of the depuration phase and analysed directly for total radioactivity. Additional water samples were also taken on days 0, 7, 14, 21 and 28 of the uptake phase and on days 3, 7 and 14 of the depuration phase. These were filtered (Whatman GF/C Glass Microfibre filter paper) prior to analysis of total radioactivity. Duplicate

aliquots of water were removed from each tank on the final 2 days of the pre-equilibration phase, on days 0, 7, 14, 21 and 28 of the uptake phase and on days 3, 7 and 14 of the depuration phase. Concentrations of [^{14}C]-Triticonazole were then determined by reversed-phase HPLC with u.v. detection.

10 fish were removed taken on days 0, 1, 3, 7, 14, 21 and 28 of the uptake phase and days 1, 3, 7, 10 and 14 of the depuration phase. After killing, fish were blotted dry, weighed and the length (from the tip of the snout to the caudal peduncle) measured. Five fish from each tank were separated into edible tissue (muscle) and non-edible tissue (viscera) and the remaining 5 fish were analysed whole. Total radioactivity in each tissue fraction was determined.

Calculations:

Concentrations of total radioactivity in whole fish and tissue fractions from all sample points and the mean concentrations of total radioactivity measured in water during the uptake phase were used to calculate the following parameters:

- Rate constants for uptake (k_1) and depuration (k_2) of test material by and from tissues.
- Bioconcentration factors, for test fish tissues and whole fish as the ratio of the uptake and depuration rate constants were calculated as follows:

Bioconcentration factor (BCF) = µg equiv. g^{-1} in tissue, C_F / µg equiv. mL^{-1} in water, C_w

- Half-life for depuration which is defined as the time taken for elimination of 50% of total radioactive residue in tissues at steady-state

Half-life = $\log_n 2 / k_2$

- Time taken to achieve 90% of the steady-state concentration in tissue (t_{90})

$t_{90} = \log_n 10 / k_2$

Statistics:

To obtain a starting value for the depuration rate constant k_2 a linear regression model was fitted to logarithmically transformed values of C_F (Concentration fish) for the depuration phase against sampling time (t). Using this k_2 value, C_w (Concentration water) and a plot of the uptake phase data, a starting value for the uptake rate constant k_1 was estimated.

This initial parameter estimates were used in the non-linear iterative regression NLIN of the statistical software package SAS Version 6.07 to determine the rate constants k_1 and k_2

The goodness of fit of the model was assessed by the correlation coefficient between the observed and fitted values the coefficient of variation of k_1 and k_2 was calculated as the percentage of the standard deviation of the parameter divided by the parameter estimate.

Results and discussion:

Exposure concentration:

A mean test concentration of 82 µg equiv./L was determined by analysis of total radioactivity with individual values ranging between 75.9 and 89.7 µg equiv./L. A mean test concentration of 88 µg/L for the uptake phase was determined by reversed-phase HPLC analysis, measured concentrations ranging from 81.3 to 91.8 µg/L. During depuration phase, concentrations were below the limit of analytical determination.

In the control tank concentrations of the test material were below limits of analytical determination throughout the test.

Analysis of total radioactivity in fish:
Concentrations of total radioactivity in fish tissues increased to 90% of the steady-state concentrations after 2.2-2.9 days of exposure to [^{14}C]-Triticonazole. The mean concentrations in each tissue after 28 days exposure were 1.172, 9.578 and 5.628 µg equiv./g for edible tissue, inedible tissue and whole fish respectively. The first-order rate constants for uptake into the edible and inedible tissue fractions were 9.50 and 97.72 ml/g/day, so that fluxes of radioactivity into inedible tissues are roughly 10-fold those into the edible fraction.
The half-life for depuration was similar for each tissue type and ranged from 0.67-0.87 days. Mean concentrations of radioactivity in edible and inedible tissue and whole fish following 14 days of depuration were 11, 23 and 11 ng equiv/g respectively. During the depuration period, tissue concentrations decreased to less than 1% of the mean concentration found at the end of the uptake phase. Therefore elimination of radioactivity from the fish was very rapid and extensive.
Tissue extracts from test fish sampled on day 14, 21 and 28 were examined by thin layer chromatography (TLC) and high performance liquid chromatography (HPLC). Principal radioactive components were triticonazole with the isomeric forms RPA 404886 and RPA 405826, RPA 406972, glucuronide conjugates of RPA 404886 and RPA 405862 and glucuronide conjugates of RPA 406972 and RPA 406341.

The results based on total radioactivity are summarised as follows:

Table 9.2-12: Rate parameters and bioconcentration factors estimated by non-linear regression analysis using a 2-compartment model

Parameter	Tissue Fraction			
	Edible	**Inedible**	**Total[a]**	**Whole Fish**
Uptake rate constant, k1 (ml/g/day)	9.5	97.72	49.07	58.2
Depuration rate constant, k2 (ml/g/day)	1.03	0.85	0.8	0.8
Bioconcentration Factor (k1/k2)	9.2	114.86	61.3	72.55
Depuration half-life (days)	0.67	0.81	0.87	0.86
Time to 90% of steady-state (days)	2.23	2.71	2.88	2.87
Correlation coefficient from model	0.57	0.88	0.90	0.90
Coefficient of variation of k1 (%)	54.1*	18.8	17.0	17.4
Coefficient of variation of k2 (%)	54.6*	19	17.2	17.6

[a]Total refers to the sum of radioactivity determined in the tissue fractions
*Model not considered to be a good fit. Results of the analysis for this tissue fraction should be treated with caution.

Table 9.2-13: Bioconcentration factors calculated directly from total radioactivity results

Day No. (Uptake Phase)	Tissue Fraction			
	Edible	**Inedible**	**Total[a]**	**Whole Fish**
1	4	60	30	28

Day No. (Uptake Phase)	Tissue Fraction			
3	9	97	49	53
7	5	89	47	68
14	12	157	83	87
21	7	112	61	94
28	14	110	61	65

[a]Total refers to the sum of radioactivity determined in the tissue fractions

Mortality was 2.5% and 0.7% in the treatment and the control, respectively. No other effects were observed throughout the study.

Conclusion:

The accumulation of radioactivity derived from exogenous [^{14}C]-triticonazole into Bluegill Sunfish tissues and its subsequent elimination followed apparent first order kinetics. Rates of elimination of radioactivity from the fish balanced those for accumulation within 3 days of initial exposure. Bioconcentration factors were 9.20, 114.86 and 72.55 for edible tissue, inedible tissue and whole fish respectively.

Triticonazole was found to comprise less than 5% of the total radioactive residue in fish after 28 days exposure. The rapid metabolism of triticonazole to highly polar conjugated products is probably the reason for the low bioaccumulation factors and rapid rates of depuration found in the study.

Comment RMS:	The study was evaluated following the recommendations of the currently valid test guideline OECD 305 (2012). **Check of validity criteria:** - The water temperature variation is less than ± 2 °C, because large deviations can affect biological parameters relevant for uptake and depuration as well as cause stress to animals. In the current study the temperature was 20.4 – 23.0°C. Fulfilled. - The concentration of dissolved oxygen did not fall below 60% saturation. In the current study the oxygen concentration was 64 – 96%. Fulfilled. - The concentration of the substance in the chambers is maintained within ± 20% of the mean of the measured values during the uptake phase. In the current study the concentration ranged between 85 % and 100.7% of the nominal concentration. Fulfilled. - The concentration of the test substance was below its limit of solubility in water. Fulfilled. - The mortality or other adverse effects/disease in both control and treated fish is less than 10% at the end of the test; where the test is extended over several weeks or months, death or other adverse effects in both sets of fish should be less than 5% per month and not exceed 30% in all. Significant differences in average growth between the test and the control groups of sampled fish could be an

indication of a toxic effect of the test chemical. In the current study mortality was 2.5% and 0.7% in the treatment and the control, respectively. Fulfilled.

In addition, the following points deviated from the test guidelines or were not reported in detail:

- According to OECD 305, uneaten food and faeces should be siphoned daily from the test chambers shortly after feeding (30 minutes to one hour). In the current test any excess food was removed after 30 min. In nine occasions, excess food was removed at other intervals after feeding but always within a relatively short period (≤ 6 h).
- According to OECD 305, for polar substances the test should be run with two or more concentrations. If only one concentration is tested, justification for the use of one concentration should be given In the current study only one concentration was tested without any justification.
- According to OECD 305, during the test, TOC should be measured in all test and control vessels. In the current study TOC was not measured.
- According to OECD 305, the pH value should be within the range of 6.0 to 8.5 at test start, but during a given test it should be within a range of ± 0.5 pH units. In the current study the pH was in a range of 7.2-8.2.
- According to OECD 305, the lipid content should be determined on the same biological material as is used to determine the concentration of the test substance at least at the start and end of the uptake phase and at the end of the depuration phase. In the current study the lipid content was not estimated.

Endpoints:

The endpoints derived from this study are:

$BCF_{Kwhole\ fish}$: 72.55

$BCF_{Kinedible}$: 114.86

$BCF_{Kedible}$: 9.2

The steady state concentration was not reported

$BCF_{whole\ fish}$: 28 – 94 (calculated directly from concentrations of ai in water and fish tissue for all days)

Conclusion of the RMS: The results of the study indicate some uncertainties as the bioconcentration factor seems to first decrease and than increase again. Furthermore some information is missing in the study report (lipid content of fish, TOC, testing of a second concentration). However, even if the validity of the study is questionable, the results have been used to be able to do a risk assessment.

Reference:	**[^{14}C]-Triticonazole bioaccumulation test in bluegill sunfish – report amendment 1**
Author(s), year:	[redacted], C.R., 1999
Report/Doc. number:	R005940
Guideline(s):	US EPA guideline 165-4
GLP:	No
Validity:	Acceptable

This amendment report has been issued to provide additional information for the [14C]-Triticonazole bioaccumulation test in bluegill sunfish by [redacted] 1996, within the following sections:

Preparation of Samples for Chromatography; solvent extraction of tissue samples, enzyme and acid hydrolysis of tissue extracts, HPLC-fish extracts and further analytical raw data and example calculations

Comment RMS:	The assessment of the concerning study was not changed by the additional information.

B.9.2.5. Acute toxicity to aquatic invertebrates

Active substance:

Reference:	**Acute Toxicity of RPA 400727 to *Daphnia magna***
Author(s), year:	Douglas, M. T., Halls, R.W.S., Macdonald, I. A., 1990b
Report/Doc. number:	R013007
Guideline(s):	OECD guideline 202 (1984)
GLP:	Yes
Deviations:	Please refer to commenting box below
Validity:	Acceptable

Material and methods:	
Test substance:	RPA 400727 purity: 99.5%, batch no.: YG2156/1
Test species:	*Daphnia magna* (Strauß); laboratory culture originating from a strain supplied by IRCHA, France
Number of organisms:	2 replicates each with 20 daphnids per treatment, control and solvent control
Age:	Young daphnids produced over night
Type of test, duration:	Static test, 48 hours
Applied concentrations:	
Nominal:	0 (control and solvent control), 1.0, 1.8, 3.2, 5.6 and 10 mg ai/L – At higher concentrations than 10 mg/L a precipitate formed immediately upon adding the solvent stock solutions to water.
Measured (mean):	Not given
Solvent:	Tween 80 - dimethylformamide
Test conditions:	
Water quality:	Dechlorinated (with sodium thiosulphate) and aged laboratory tap water. Total hardness = 350 mg/l as $CaCO_3$, alkalinity: not given, conductivity: not given
Temperature:	21°C (0 - 48 h)
pH:	8.1 – 8.4 (0 - 48 h)
O_2 content:	94.5 – 97.9 % saturation (8.2 – 8.5 mgO_2/L)
Light regime:	16 hours light / 8 hours darkness
Test parameters:	Immobility was assessed after 24 and 48 hours. During the exposure the daphnids were not fed. Measurements of pH, temperature and dissolved oxygen concentrations were made at the start and end of the test. Temperature was recorded after at the start of the test, after 24 hours and after 48 hours.

For chemical analysis of RPA 400727 in the test media samples were taken at test initiation (0 h) and termination (48 h).

Statistics: The EC_{50}, and 95 % confidence limits were calculated using Thompson, W.R. and Weil, C.S. (1952).

Findings:

Analytical data: The mean measured concentrations are in a range of 92 and 106% of nominal test concentrations. Hence, the endpoint is based on nominal concentrations.

Effects:

Table 9.2-14: Effects on daphnids (*D. magna*) exposed to RPA 400727

Triticonazole [mg ai/L] (nominal)	Mean cumulative immobilized organisms [%]	
	24 hours	48 hours
Control	0	0
Solvent control	0	0
1.0	0	0
1.8	0	0
3.2	0	5
5.6	15	30
10.0	45	65
48 h EC_{50} = 9 mg ai/L (95 % C.I. 6.0 – 14.0 mg ai/L) 48 h NOEC = 3.2 mg ai/L Based on nominal concentrations		

Conclusion: The 48-hour EC_{50} was calculated as 9 mg/L based on nominal concentrations.

Comment RMS: The study was evaluated following the recommendations of the currently valid test guidelines OECD 202 (2004)

Check of validity criteria:

- In the control, including the control containing the solubilising agent, not more than 10 per cent of the daphnids should hve been immobilised or show other signs of disease or stress (e.g. discoloration or unusual behaviour). Neither in the control nor in the solvent control immobilisation occurred. Fulfilled.
- The the dissolved oxygen concentration at the end of the test should be ≥ 3 mg/L in control and test vessels. The dissolved oxygen concentration was between 8.2 and 8.4 mg/L after 48 hours. Fulfilled.

In addition, the following points deviated from the test guidelines or were not reported in detail:

- OECD 202 recommends testing of at least 20 daphnids per test group divided into four groups of five animals. In the study, 20 daphnids in divided in two groups of ten animals were tested.
- OECD 202 recommends testing with a reference substance every month and at least twice a year. No information about reference testing is given in the study

report.

- OECD 202 recommends a hardnes of the dilution water between 140 and 250 mg/L as CaCO3. The hardness of the water in the test was a pprox. 350 mg/L as $CaCO_3$.

Acceptability of the analytical methods used in the test: Evaluation of the analytical methods (water/sediment/soil) according to the Guidance Document SANCO/3029/99 rev. 4.

Method: HPLC

Linearity: Calibration is based on duplicate determination at three concentrations. The calibration function was within the range from 1.11 – 13.87 of (± 20% of nominal concentration), no information on linearity and no regression parameter is given.

Accuracy: 3fortifications levels (each 2 and 3 measurements, respectively): 1 x LOQ and 10 x LOQ should be measured. The LOQ was not explicitly given, however measurements were conducted at 0.988 mg/L and at the tenfold (9.977 mg/L). Mean recoveries for each level: 101-105% which is in the recommended range of 70-110%

Precision: The relative standard deviation per fortification level ≤ 20 %

LOQ: The limit of quantification (LOQ) was not explicitly given, however the lowest concentration measured was 0.0988 mg/L

LOD: The limit of detection (LOD) = 0.037 mg/L

The analytical method fulfils the requirements of guideline SANCO/3029/99 rev. 4.

Endpoints:

The NOEC given in the study report is based on 3.2 mg ai/L, showing 5 % effect. As the effects show a dose response, the NOEC is set at the lower level of 1.8 mg/L where no effect was observed.

EC_x values were recalculated with ToxRatPro® v.3.2.

EC_{10} = 3.86 mg ai/L nominal concentration (95% C.I.: 2.40-4.88 mg ai/L) reliability based on normalised widths of C.I. = good

EC_{20} = 4.92 mg ai/L nominal concentration (95% C.I.: 3.55-6.04 mg ai/L)

EC_{50} = 7.85 mg ai/L nominal concentration (95% C.I.: 6.40-10.71 mg ai/L)

NOEC = 1.8 mg ai/L nominal concentration

Conclusion of the RMS: Based on the evaluation of the study the daphnid acute toxicity test is considered valid.

Reference:	**Triticonazole technical – acute toxicity to mysids (*Mysidopsis bahia)* under flow-through conditions**
Author(s), year:	Sousa, J.V., 1998d
Report/Doc. number:	B004421
Guideline(s):	FIFRA Guideline 72-3
GLP:	Yes
Deviations:	Please refer to commenting box below
Validity:	Acceptable

Material and methods:	
Test substance:	Triticonazole technical (RPA 400727), batch no.: OP9750057, purity: 90.52%
Test species:	Mysid shrimp (*Americamysis bahia*, formerly known as *Mysidopsis bahia*); Springborn laboratory culture
Number of organisms:	2 replicates each with 10 mysid shrimp per treatment, control and solvent control
Age:	≤ 24 hours
Type of test, duration:	Flow-through test, 96 hours; 90% test solution replacement rate of approx. 9 hours
Applied concentrations:	
Nominal:	
Range-finding	0 (control and solvent control), 0.65, 1.1, 1.8, 3.0 and 5.0 mg ai/L
Definitive exposure #1	0 (control and solvent control), 0.64, 1.1, 1.8, 3.0, and 4.9 mg ai/L
Definitive exposure #2	0 (control and solvent control), 1.0, 1.7, 2.8, 4.7 and 7.9 mg ai/L
Mean measured:	
Definitive exposure #1	0 (control and solvent control), 0.6, 1.0, 1.7, 2.9 and 4.6 mg ai/L
Definitive exposure #2	0 (control and solvent control), 1.1, 1.8, 2.9, 4.9 and 7.5 mg ai/L
Solvent:	Acetone
Test conditions:	
Water quality:	Natural filtered sea water , salinity 31 - 33 $^{o}/_{oo}$, TOC concentration < 2.0 mg/L
Temperature:	24 - 25 °C
pH:	7.8 – 8.0 (0 - 96 h)
O_2 content:	5.2 – 7.9 mg/L (76 - 115 % saturation)
Light regime:	16 hours light / 8 hours darkness, 240 to 1100 lux
Test parameters:	Mortality and sublethal effects were assessed after 0, 24, 48, 72 and 96 hours. Measurements of pH, temperature and dissolved oxygen concentrations were made once daily throughout each exposure period. Temperature was additionally monitored continuously.
Analytical measurements:	Samples from both replicates of the high, middle, and low treatment level solutions, the dilution water control solution were taken prior to the start of the first and the second definitive exposure, at test initiation (0 hour) and test termination (96 hours).

Statistics: The EC_{50} value and 95% confidence limits were calculated by a computer program (Stephan, 1982, personal communication) with three statistical methods (moving average angle analysis, probit analysis and nonlinear interpolation with 95% confidence limits calculated by binomial probability).

Findings:

Analytical measurements:

Definitive exposure #1 The analytical data indicated that the mean measured triticonazole concentrations were between 93 and 98 % of the nominal test concentrations. However, the results are based on mean measured concentrations.

Definitive exposure #2 The analytical data indicated that the mean measured triticonazole concentrations were between 95 and 110 % of the nominal test concentrations. However, the results are based on mean measured concentrations.

Effects: Following 96 hours of exposure, 40%, 50% 70% and 70% mortality was observed

Range-finding test: in the four highest treatment levels, 1.1, 1.8, 3.0 and 5.0 mg ai/L, respectively

Table 9.2-15: Effects on mysid shrimp (*Americamysis bahia*) exposed to technical triticonazole #1

Triticonazole [mg ai/L] (mean measured)	**Mean cumulative mortality [%]**			
	24 hours	**48 hours**	**72 hours**	**96 hours**
Control	0	0	0	0
Solvent control	0	0	0	5
0.6	0	0	0	10
1.0	0	0	0	20
1.7	0	0	25^{ac}	55^{b}
2.9	0^{ab}	20^{a}	40^{abc}	40^{abc}
4.6	10^{b}	30^{ab}	45^{ab}	50^{abc}
96 h $LC_{50} \geq 1.7$ mg ai/L 96 h NOEC = 1.0 mg ai/L				

[a] partial loss of equilibrium, [b] lethargic, [c] complete loss of equilibrium

Table 9.2-16: Effects on mysid shrimp (*Americamysis bahia*) exposed to technical triticonazole #2

Triticonazole [mg ai/L] (mean measured)	**Mean cumulative mortality [%]**			
	24 hours	**48 hours**	**72 hours**	**96 hours**
Control	0	0	0	0
Solvent control	0	0	0	0
1.1	0	0	25^{b}	30^{a}
1.8	0	0^{b}	50^{b}	60^{bcd}
2.9	5^{bd}	5^{bd}	30^{abcc}	65^{ac}
4.9	15^{be}	20^{b}	50^{abc}	55^{abc}
7.5	35^{abc}	40^{c}	85^{c}	85^{c}
96 h LC_{50} = 1.9 mg ai/L (95% C.I. 0.68 – 3 mg ai/L) 96 h NOEC < 1.1 mg ai/L				

[a] partial loss of equilibrium, [b] lethargic, [c] complete loss of equilibrium, [d] erratic swimming, [e] lethargic at surface of test solution

Conclusion: The 96 hour LC_{50} of triticonazole technical to mysid shrimp, *Americamysis bahia* was determined under the flow-through test conditions of this study and is 1.9 mg/L (95% C.I. 0.68 - 3 mg/L), based on mean measured concentrations. The NOEC is 1 mg/L.

Comment RMS: The study was evaluated following the recommendations of the currently valid test guidelines OCSPP 850.1035 (2016)

Check of validity criteria:

- All test vessels are identical. Fulfilled.
- Treatments are randomly or indiscriminately assigned to individual tes vessel locations, individual test organisms were randomly or indiscriminately assigned to test vessels or compartments. Fulfilled.
- A dilution water control (and vehicle (solvent) control, if a vhicle was used) was included in the test. A dilution water control and a solvent control with acetone were tested in the study. Fulfilled.
- Not more than 10% of the organisms in either the dilution water or vehicle (solvent) controls showed signs of disease, stress (e.g., discoloration, unusual behavior, immobilization), and/or death. During the test, no immobilisation occurred in the control or solvent control. Fulfilled.
- No surfactant or dispersant was used in the preparation of a stock or test solution. (However, adjuvants may be used when testing pesticide typical end-use products). Fulfilled

In addition, the following points deviated from the test guidelines or were not reported in detail:

- OCSPP 850.1035 recommends testing of a series of widely-spaced concentrations in the range finding test, e.g. 1, 10, 100 mg/L. The range finding test in this study was testet with concentrations between 0.6 and 5.0 mg/L.
- According to OCSPP 850.1035 dissolved oxygen in the dilution water prior to use in a test should be between 90 and 100% saturation. Dissolved oxygen saturation prior to test was not reported. During the test the saturation was between 76 and 115 %.
- According to OCSPP 850.1035 light intensity should range from 540 to 1080 lux. The light intensity during the study ranged from 240 to 1100 lux.

Acceptability of the analytical methods used in the test: The evaluation of the analytical methods was conducted according to the Guidance Document SANCO/3029/99 rev. 4. Based on the evaluation (see Volume 3 B.5-CA, B.5.1.2.6) the analytical method is considered valid to quantify the amount of

	triticonazole in fresh and seawater. LOQ: = 0.148 mg/L **Endpoints:** The RMS agrees on the endpoints given in the study report. LC_{50} = 1.9 mg ai/L (C.I. 95% = 0.68 – 3 mg/L) $LC_{50} \geq$ 1.7 mg ai/L NOEC = 1 mg ai/L Based on mean measured concentration **Conclusion of the RMS:** Based on the evaluation of the study the mysid acute toxicity test is considered valid.

Reference:	**Triticonazole technical – acute toxicity to eastern oyster (*Crassostrea virginica*) under flow-through conditions**
Author(s), year:	Dionne, E. 1998a
Report/Doc. number:	SLI Report No.: 98-5-7331; C019777
Guideline(s):	FIFRA Guideline 72-3
GLP:	Yes
Deviations:	Please refer to commenting box below
Validity:	Acceptable

Material and methods:	
Test substance:	Triticonazole technical (RPA 400727), batch no.: OP9750057, purity: 90.52%
Test species:	Eastern oyster (*Crassostrea virginica*); P. Cummins Oyster Co., Annapolis, Maryland
Number of organisms:	Range-finding test: 1 replicate with 20 oysters per treatment; Definitive test: 2 replicates, each with 10 oysters per treatment, control and solvent control
Age:	Reproductively immature; mean valve height of 42 (± 4.6) mm
Type of test, duration:	Flow-through test, 96 hours, approx. 6 solution volume replacements per day
Applied concentrations:	
Range-finding test	
Nominal:	0 (control and solvent control), 1.3, 2.5, 5.0, 10 and 20 mg ai/L
Definitive test	
Nominal:	0 (control and solvent control), 1.3, 2.1, 3.5, 5.9 and 9.9 mg ai/L
Mean measured:	0 (control and solvent control), 1.4, 2.2, 3.3, 4.9 and 8.3 mg ai/L
Solvent:	Acetone
Test conditions:	
Water quality:	Natural unfiltered seawater, salinity 31 - 32 $^{o}/_{oo}$, TOC < 2.0 mg/L

Temperature:	20 - 22 °C
pH:	7.3 – 7.8 (0 - 96 h)
O_2 content:	4.4 – 7.0 mg/L (61 - 93 % saturation)
Light regime:	16 hours light / 8 hours darkness
Feeding:	During the exposure the oysters received supplemental feedings of algae.
Test parameters:	Effects on shell deposition were assessed after 96 hours. Measurements of pH, temperature and dissolved oxygen concentrations and salinity were made at each 24-hour interval of the definitive exposure. Temperature was additionally monitored continuously in one replicate of the dilution water control.
Analytical measurements:	Samples of both replicates of the high, middle and low treatment levels and the dilution water control were taken immediately prior to the initiation of the definitive test. At 0 and 96 hours samples were taken from each replicate test solution and the controls.
Statistics:	The 96-hour EC_{50} value based on reduction of shell deposition (growth) and 95% confidence limits were determined by inverse prediction (Sokal and Rohlf, 1981) The NOEC was determined by using Williams' Test (Williams, 1971, 1972).

Findings:

Analytical measurements:	The analytical data indicated that the mean measured triticonazole concentrations were between 84 and 100 % of the nominal test concentrations. However, the results are based on mean measured concentrations.
Effects:	
Range-finding test	At test termination, shell growth reduction of 10, 0, 0, 12 and 41% was observed for oysters exposed to nominal concentrations of 1.3, 2.5, 5.0, 10 and 20 mg ai/L
Definitive test	At test termination one mortality was observed among oysters exposed to both the 1.4 mg ai/L treatment level and the solvent control.

Table 9.2-17: Effects on eastern oyster (*Crassostrea virginica*) exposed to technical triticonazole

Triticonazole [mg ai/L] (mean measured)	**Mean shell Deposition[a] [mm]**	**Mean percent reduction[b]**
Control	2.0 (0.9)	NA
Solvent control	1.8 (0.8)	NA
Pooled control	1.9 (0.9)	NA
1.4	1.8 (0.8)	6.9
2.2	1.5 (0.8)	22[c]
3.3	1.4 (0.8)	26[c]
4.9	1.2 (0.8)	35[c]
8.3	0.94 (0.7)	50[c]
96 h EC_{50} = 8.9 mg ai/L (95% C.I. 5.6 – 17 mg ai/L) 96 h NOEC = 1.4 mg ai/L		

[a] mean shell deposition is presented with the standard deviation in parentheses and represents the measurements of 40 oysters per treatment and control.

[b] Calculated values are based on actual analytical results and not on the rounded values (two significant figures) presented in this table

NA = not applicable

[c] Significantly reduced compared to the pooled control.

Conclusion: The 96 hour EC_{50} of triticonazole technical to eastern oyster, *Crassostrea virginica* was determined under the flow-through test conditions of this study it is 8.9 mg/L (95% C.I. 5.6 - 17 mg/L) based on mean measured concentrations. The NOEC is 1.4 mg/L.

Comment RMS: The study was evaluated following the recommendations of the currently valid test guidelines OCSPP 850.1025 (2016)

Check of validity criteria:

- All test vessels are identical. Fulfilled.
- Treatments are randomly or indiscriminately assigned to individual tes vessel locations, individual test organisms were randomly or indiscriminately assigned to test vessels or compartments. Fulfilled.
- A dilution water control (and vehicle (solvent) control, if a vhicle was used) was included in the test. A dilution water control and a solvent control with acetone were tested in the study. Fulfilled.
- Not more than 10% of the organisms in either the dilution water or vehicle (solvent) controls showed signs of disease, stress (e.g., shell gaping, excessive mucus), and/or death. During the test, one mortality occurred in the solvent control. Fulfilled
- No surfactant or dispersant was used in the preparation of a stock or test solution. (However, adjuvants may be used when testing pesticide typical end-use products). Fulfilled.

In addition, the following points deviated from the test guidelines or were not reported in detail:

- OCSPP 850.1025 recommends testing of a series of widely-spaced concentrations in the range finding test, e.g. 1, 10, 100 mg/L. The range finding test in this study was testet with concentrations between 1.3 and 20 mg/L.
- According to OCSPP 850.1025 dissolved oxygen in the dilution water prior to use in a test should be between 90 and 100% saturation. Dissolved oxygen saturation prior to test was not reported. During the test the saturation was between 76 and 115 %.
- According to OCSPP 850.1025 the pH should be between 7.5 and 8.5. The pH in the study ranged between 7.3 and 7.8.
- According to OCSPP 850.1025 light intensity should range from 540 to 1080 lux. The light intensity was not reported in this study.

	Acceptability of the analytical methods used in the test: The evaluation of the analytical methods was conducted according to the Guidance Document SANCO/3029/99 rev. 4. Based on the evaluation (see Volume 3 B.5-CA, B.5.1.2.6) the analytical method is considered valid to quantify the amount of triticonazole in fresh and seawater. LOQ: = 0.148 mg/L **Endpoints:** The RMS agrees on the endpoints given in the study report. EC_{50} = 8.9 mg ai/L mean measured concentration NOEC = 1.4 mg ai/L mean measured concentration **Conclusion of the RMS:** Based on the evaluation of the study the oyster acute toxicity test is considered valid.

Reference:	**Experimental assessment of the environmental fate and effects of triazoles and benzotriazloe**
Author(s), year:	Durjava, M.K. et al., 2013a
Report/Doc. number:	BASF DocID 2015/1177633
Guideline(s):	None
GLP:	No

Material and methods:

Test substance:	Triticonazole (BAS 595 F), CAS no.: 131983-72-7; purchased from Fluka® Analytical (Sigma-Aldrich).
Test species:	Water flea (*Daphnia magna* STRAUS), < 24 h old at test initiation
Test design:	Static system (48 hours), minimum of 5 test concentrations plus control, 4 replicates with 5 daphnids in each; assessment of immobility after 48 hours.
Test concentrations:	Range of triticonazole concentrations (5 concentrations at least).
Test conditions:	100 mL glass beakers, test volume 20 mL; dilution water "M4-medium"; substances were dissolved in dimethyl sulphoxide (DMSO); pH, oxygen content and temperature not reported; dissolved oxygen levels and pH did not change significantly during the experiments.
Test parameters:	EC_{50} based on immobility of daphnids.
Analytics:	Chemical measurements of test item concentrations was conducted using a GC-method with MS detection
Statistics:	Descriptive statistics; non-linear regression curve fitting assuming a sigmoid dose–response curve for EC_{50} calculation.

Findings:

Analytical data:	Analytical measurements: Chemical measurements of test item concentrations in the test cultures was conducted at the beginning and at the end of the test. The following biological results are based on nominal concentrations.
Biological findings:	The 48 h EC_{50} value for *D. magna* was determined to be 9.56 mg/L (95% confidence limits: 5.70 - 16.0 mg/L).
Conclusion:	In a 48-hour static acute toxicity study with Daphnia magna the EC_{50} of triticonazole was 9.56 mg ai/L based on nominal concentrations.

Comment RMS:	The EC_{50} for Daphnia magna estimated in this public literature study is within the range of the EC_{50} determined in the studies conducted for regulatory purpose.

Metabolites:

Reference:	**Acute Toxicity of RPA 404766 to *Daphnia magna***
Author(s), year:	Sewell, I. G., Mullee, D.M. 2001b
Report/Doc. number:	C017902
Guideline(s):	OECD guideline 202 (1984)
GLP:	Yes
Deviations:	Please refer to commenting box below
Validity:	Acceptable

Material and methods:

Test substance:	RPA 404766 (Reg.No. 5079285) purity: 969g/kg, batch no.: BESS0540
Test species:	*Daphnia magna*; in-house laboratory culture
Number of organisms:	Range-finding test: 1 replicate with 10 daphnids per treatment and control Definitive test: 4 replicates each with 10 daphnids per treatment and control
Age:	1st instar larvae, <24 hours
Type of test, duration:	Range-finding test: static Definitive test: Semi-static test, media renewal after 24 hours and 48 hours
Applied concentrations:	
Range-finding study:	
Nominal	0 (control), 0.1, 1.0, 10 and 100 mg/L
Definitive study – limit test	
Nominal:	0 (control), 100 mg /L
Measured (mean):	Not given
Solvent:	In the text of the study report a solvent control is mentioned once, but no values for a solvent control were reported. The solvent control in the text is assumed to be mentioned by mistake.
Test conditions:	
Water quality:	Reconstituted water was used to maintain the stock animals; approx. theoretical hardness 250 mg/L as $CaCO_3$
Temperature:	21°C (0 - 48 h)
pH:	8.0 (0 - 48 h)
O_2 content:	Range-finding test: 91 – 94 % saturation (8.1 – 8.4 mg O_2/L) Definitive test: 90-93 % saturation (8.0 – 8.3 mg O_2/L)
Light regime:	16 hours light / 8 hours darkness with 20 minute dawn and dusk transition periods
Feeding:	During the exposure the daphnids were not fed.
Test parameters:	Immobilisation or adverse reactions to exposure were recorded at 24 and 48 hours

after start of exposure.

Measurements of pH, temperature and dissolved oxygen concentrations were recorded daily throughout the study.

For chemical analysis of RPA 404766 in the test media samples were taken from the control and the 100 mg/L test group at 0 (fresh media), 24 (old and fresh media) and 48 hours (old media).

Statistics: No statistical analysis was carried out since no daphnid died in the highest tested concentration.

Findings:

Analytical data: The analytical data indicated that the RPA 404766 concentrations were maintained within 20% of nominal throughout the duration of the study.

The mean measured concentrations are in a range of 97 and 103% of nominal test concentrations. Hence, the endpoint is based on nominal concentrations.

Effects:

Range finding study:

Table 9.2-18: Effects on daphnids (*D. magna*) exposed to RPA 404766 range- finding study

RPA 404766 [mg /L] (nominal)	Cumulative immobilized organisms [%]	
	24 hours	48 hours
Control	0	0
0.1	0	0
1.0	0	0
10	0	0
100	0	0

Table 9.2-19: Effects on daphnids (*D. magna*) exposed to RPA 404766 definitive test

RPA 404766 [mg /L] (nominal)	Cumulative immobilized organisms [%]	
	24 hours	48 hours
Control	0	0
100	0	0
48 h EC_{50} > 100 mg /L based on nominal concentration NOEC = 100 mg/L based on nominal concentration		

Conclusion: The acute toxicity of RPA404766 to *Daphnia magna* has been investigated.

The 48-hour EC_{50} was calculated as > 100 mg/L based on nominal concentrations.

Comment RMS: The study was evaluated following the recommendations of the currently valid test guidelines OECD 202 (2004)

Check of validity criteria:

- In the control, including the control containing the solubilising agent, not more

than 10 per cent of the daphnids should hve been immobilised or show other signs of disease or stress (e.g. discoloration or unusual behaviour). During the test, no immobilisation occurred in the control. Fulfilled.

- The the dissolved oxygen concentration at the end of the test should be ≥ 3 mg/L in control and test vessels. The dissolved oxygen concentration was between 8.0 and 8.3 mg/L during the test. Fulfilled.

In addition, the following points deviated from the test guidelines or were not reported in detail:

- OECD 202 recommends testing with a reference substance every month and at least twice a year. No information about reference testing is given in the study report.
- OECD 202 recommends a hardnes of the dilution water between 140 and 250 mg/L as $CaCO_3$. The hardness of the water in the test was not measured.

Acceptability of the analytical methods used in the test: Evaluation of the analytical methods (water/sediment/soil) according to the Guidance Document SANCO/3029/99 rev. 4.

Method: HPLC

Linearity: Calibration is based on determination at ten concentrations. No information is given about the number of replicate measurements.

The calibration function was linear within a range from 0 – 100 mg/L (0 to 200% of the working concentration. Linearity was confirmed with $r^2 = 1$, ranging from 0 to 100 mg/L.

Accuracy: 5 fortification levels in a range of 87 to 132 mg/L measured. No information is given about the number of replicate measurements.

1 x LOQ (1.1 mg/L) is not included in the measurements.

Mean recoveries for each level: 90-105%

Precision: No information given about precision

LOQ: = 1.1 mg/L

LOD: = not given

The analytical method fulfils the requirements of guideline SANCO/3029/99 rev. 4.

Endpoints:

The RMS agrees on the Endpoints given in the study report.

EC_{50} > 100 mg /L nominal concentration

NOEC = 100 mg/L nominal concentration

Conclusion of the RMS: Based on the evaluation of the study the daphnid acute toxicity test is considered valid.

Reference:	**Acute Toxicity of RPA 407922 to *Daphnia magna***
Author(s), year:	Sewell, I. G., Mullee, D.M. 2001a
Report/Doc. number:	1392/022; C017901
Guideline(s):	OECD guideline 202 (1984)
GLP:	Yes
Deviations:	Please refer to commenting box below
Validity:	Acceptable

Material and methods:

Test substance:	RPA 407922 (Reg.No. 5079288) purity: 995g/kg, batch no.: BESS521B
Test species:	*Daphnia magna*; in-house laboratory culture
Number of organisms:	Range-finding test: 1 replicate with 10 daphnids per treatment and control Definitive test: 4 replicates each with 10 daphnids per treatment and control
Age:	1st instar larvae, <24 hours
Type of test, duration:	Range-finding test: static Definitive test: Semi-static test, media renewal after 24 hours; 48 hours
Applied concentrations:	
Range-finding study:	
Nominal	0 (control), 0.01, 0.1, 1.0, 10 and 100 mg/L
Definitive study – limit test	
Nominal:	0 (control), 100 mg /L
Measured (mean):	Not given
Solvent:	None
Test conditions:	
Water quality:	Reconstituted water was used to maintain the stock animals; approx. theoretical hardness 250 mg/L as $CaCO_3$
Temperature:	21°C (0 - 48 h)
pH:	Range-finding test: 7.5 – 8.0 (0 - 48 h) Definitive test: 7.4 – 7.9 (0-48 h)
O_2 content:	Range-finding test: 92 – 94 % saturation (8.2 – 8.4 mg O_2/L) Definitive test: 92-96 % saturation (8.2 – 8.5 mg O_2/L)
Light regime:	16 hours light / 8 hours darkness with 20 minute dawn and dusk transition periods
Test parameters:	Immobilisation or adverse reactions to exposure were recorded at 24 and 48 hours after start of exposure. During the exposure the daphnids were not fed. Measurements of pH, temperature and dissolved oxygen concentrations were recorded daily throughout the study. For chemical analysis of RPA 407922 in the test media samples were taken from

the control and the 100 mg/L test group at 0 (fresh media), 24 (old and fresh media) and 48 hours (old media).

Statistics: No statistical analysis was carried out since no daphnid died in the highest tested concentration

Findings:

Analytical data: The analytical data indicated that the RPA 404766 concentrations were maintained within 20% of nominal throughout the duration of the study.
The mean measured concentrations are in a range of 100 and 104% of nominal test concentrations. Hence, the endpoint is based on nominal concentrations.

Effects:

Range finding study:

Table 9.2-20: Effects on daphnids (*D. magna*) exposed to RPA 407922 - range-finding study

RPA 407922 [mg /L] (nominal)	Cumulative immobilized organisms [%]	
	24 hours	48 hours
Control	0	0
0.01	0	0
0.1	0	0
1.0	0	0
10	0	0
100	0	0

Table 9.2-21: Effects on daphnids (*D. magna*) exposed to RPA 407922 - definitive test

RPA 407922[mg /L] (nominal)	Cumulative immobilized organisms [%]	
	24 hours	48 hours
Control	0	0
100	0	0
48 h EC_{50} > 100 mg /L based on nominal concentration NOEC = 100 mg/L based on nominal concentration		

Conclusion: The acute toxicity of RPA 407922 to *Daphnia magna* has been investigated.
The 48-hour EC_{50} was calculated as > 100 mg/L based on nominal concentrations.

Comment RMS: The study was evaluated following the recommendations of the currently valid test guidelines OECD 202 (2004)

Check of validity criteria:

- In the control, including the control containing the solubilising agendt, not more than 10 per cent of the daphnids should hve been immobilised or show other signs of disease or stress (e.g. discoloration or unusual behaviour). During the test, no immobilisation occurred in the control. Fulfilled.

- The the dissolved oxygen concentration at the end of the test should be ≥ 3 mg/L in control and test vessels. The dissolved oxygen concentration was between 8.2 and 8.5 mg/L during the test. Fulfilled.

In addition, the following points deviated from the test guidelines or were not reported in detail:

- OECD 202 recommends testing with a reference substance every month and at least twice a year. No information about reference testing is given in the study report.
- OECD 202 recommends a hardnes of the dilution water between 140 and 250 mg/L as CaCO3. The hardness of the water in the test was not measured.

Acceptability of the analytical methods used in the test: Evaluation of the analytical methods (water/sediment/soil) according to the Guidance Document SANCO/3029/99 rev. 4.

Method: HPLC

Linearity: Calibration is based on determination at seven concentrations in a range of 5.4 to 110 mg/L. No information is given about the number of replicate measurements.

The calibration function was linear within the range from 5.4 – 100 mg/L (11% to 220% of the working concentration) with $r^2 = 1$.

Accuracy: One fortification level (105 mg/L) with 5 measurements. The measurement does not include 1 x LOQ (5.4 mg/L).

Mean recovery: 99%

Precision: The relative standard deviation per fortification level ≤ 20 %

LOQ: = 5.4 mg/L

LOD: = not given

The analytical method fulfils the requirements of guideline SANCO/3029/99 rev. 4.

Endpoints:

The RMS agrees on the endpoints given in the study report.

EC_{50} > 100 mg /L nominal concentration

NOEC = 100 mg/L nominal concentration

Conclusion of the RMS: Based on the evaluation of the study the daphnid acute toxicity test is considered valid.

Reference:	**Acute Toxicity of RPA 406341 to *Daphnia magna***
Author(s), year:	Sewell, I. G., Mullee, D.M. 2002a
Report/Doc. number:	C020498
Guideline(s):	OECD guideline 202 (1984)
GLP:	Yes
Deviations:	Please refer to commenting box below
Validity:	Acceptable

Material and methods:

Test substance:	RPA 406341 (Reg.No. 5059144) Purity94.7%, batch no.: MD 1994
Test species:	*Daphnia magna*; in-house laboratory culture
Number of organisms:	Range-finding test: 1 replicate with 10 daphnids per treatment and control Definitive test: 2 replicates each with 10 daphnids per treatment and control
Age:	1st instar larvae, <24 hours
Type of test, duration:	Range-finding test: not stated Definitive test: Semi-static test, media renewal after 24 hours; 48 hours
Applied concentrations:	
Range-finding study:	
Nominal:	0 (control), 0.1, 1.0, 10, 100 mg/L
Definitive study – limit test	
Nominal:	0 (control), 1.0, 3.2, 5.6, 10, 18, 32, 56 and 100 mg/L
Measured (mean):	Not given
Solvent:	None
Test conditions:	
Water quality:	Reconstituted water as used to maintain the stock animals; approx. theoretical hardness 250 mg/L as $CaCO_3$
Temperature:	21°C (0 - 48 h)
pH:	Range-finding test: not reported Definitive test: 7.8 – 8.0 (0-48 h)
O_2 content:	Range-finding test: not reported Definitive test: 90-94 % saturation (8.0 – 8.4 mg O_2/L)
Light regime:	16 hours light / 8 hours darkness with 20 minute dawn and dusk transition periods
Feeding:	During the exposure the daphnids were not fed.
Test parameters:	Immobilisation or adverse reactions to exposure were recorded at 24 and 48 hours after start of exposure. Measurements of pH, temperature and dissolved oxygen concentrations were recorded daily throughout the study.

For chemical analysis of RPA 406341 in the test media samples were taken from the control and the 100 mg/L test group at 0 (fresh media), 24 (old and fresh media) and 48 hours (old media).

Statistics: The EC_{50} value and associated confidence limits at 48 hours were calculated by the trimmed Spearman-Karber method (Hamilton et al 1977) using ToxCalc computer software package (ToxCalc 1999).

Findings:

Analytical data: The analytical data indicated that the RPA 406431 concentrations were maintained within 20% of nominal throughout the duration of the study.

The mean measured concentrations are in a range of 104 and 114% of nominal test concentrations. Hence, the endpoint is based on nominal concentrations.

Effects:

Range finding study:

Table 9.2-22: Effects on daphnids (*D. magna*) exposed to RPA 406341 - range-finding study

RPA 406341 [mg /L] (nominal)	**Cumulative immobilized organisms [%]**	
	24 hours	**48 hours**
Control	0	0
0.1	0	0
1	0	0
10	0	0
100	0	100

Table 9.2-23: Effects on daphnids (*D. magna*) exposed to RPA 406341 - definitive test

RPA 406341[mg /L] (nominal)	**Cumulative immobilized organisms [%]**	
	24 hours	**48 hours**
Control	0	0
1.0	0	0
1.8	0	0
3.2	0	0
5.6	0	0
10	0	0
18	0	0
32	0	0
56	0	70
100	15	100
48 h EC_{50} = 50 mg /L based on nominal concentration NOEC = 32 mg/L		

Conclusion: The acute toxicity of RPA 406341to *Daphnia magna* has been investigated.

The 48-hour EC_{50} was calculated as 50 mg/L based on nominal concentrations.

Comment RMS:	The study was evaluated following the recommendations of the currently valid test guidelines OECD 202 (2004) **Check of validity criteria:** - In the control, including the control containing the solubilising agent, not more than 10 per cent of the daphnids should hve been immobilised or show other signs of disease or stress (e.g. discoloration or unusual behaviour). During the test, no immobilisation occurred in the control. Fulfilled. - The the dissolved oxygen concentration at the end of the test should be ≥ 3 mg/L in control and test vessels. The dissolved oxygen concentration was between 8.0 and 8.4 mg/L during the test. Fulfilled. **In addition, the following points deviated from the test guidelines or were not reported in detail:** - OECD 202 recommends testing of at least 20 daphnids per test group divided into four groups of five animals. In the study, 20 daphnids in divided in two groups of ten animals were tested. - OECD 202 recommends testing with a reference substance every month and at least twice a year. No information about reference testing is given in the study report. - OECD 202 recommends a hardness of the dilution water between 140 and 250 mg/L as $CaCO_3$. The hardness of the water in the test was not measured. **Acceptability of the analytical methods used in the test:** Evaluation of the analytical methods (water/sediment/soil) according to the Guidance Document SANCO/3029/99 rev. 4. Method: HPLC Linearity: Calibration is based on determination at seven concentrations in a range of 0 to 100 mg/L. No information is given about the number of replicate measurements. The calibration function was linear within the range from 0 – 100 mg/L (0% to 200% of the working concentration) with $r^2 = 0.9999$. Accuracy: Three fortification levels with 5 measurements each. The measurement does not include 1 x LOQ (0.0089 mg/L) and 10 x LOQ (0.089 mg/L). Mean recovies : 90 and 101% Precision: The relative standard deviation per fortification level ≤ 20 % LOQ: = 0.0089 mg/L LOD: = not given The analytical method fulfils the requirements of guideline SANCO/3029/99 rev. 4. **Endpoints:**

	The RMS recalculated the endpoints with ToxRat Pro® v. 3.1. EC_{10} = 34.70 mg/L nominal concentration (95% C.I.: 27.40-39.90 mg/L) reliability based on normalised width of C.I. = good EC_{20} = 40.70 mg/L nominal concentration (95% C.I.: 34.13-45.75 mg/L) EC_{50} = 51.78 mg/L nominal concentration (95% C.I.: 58.00-46.11 mg/L) NOEC = 32 mg/L nominal concentration **Conclusion of the RMS:** Based on the evaluation of the study the daphnid acute toxicity test is considered valid.

Reference:	**Acute Toxicity of RPA 406203 to Daphnids (*Daphnia magna)* under Flow-through Conditions**
Author(s), year:	Putt, E., 1998a
Report/Doc. number:	C044320
Guideline(s):	FIFRA Guideline 72-2
GLP:	Yes
Deviations:	Please refer to the commenting box below
Validity:	Acceptable

Material and methods:

Test substance:	RPA 406203 (Reg.No. 5079359) Purity 99.8.g/kg, batch no.: OB0012
Test species:	*Daphnia magna*; Springborn culture facility
Number of organisms:	Range-finding test: not reported Definitive test: 2 replicates each with 10 daphnids per treatment, control and solvent control
Age:	≤ 24 hours
Type of test, duration:	Range-finding test: flow-through, 24-hours Definitive test: flow-through, 48 hours; each replicate vessel received an average of 10 solution volume replacements per day (volume replacement rate of approx. 5.5 hours)

Applied concentrations:

Range-finding study (24-hours): Nominal:	0 (control and solvent control), 1.3, 2.2, 3.6, and 10 mg/L
Definitive study – limit test Nominal:	0 (control and solvent control), 1.3, 2.2, 3.6, 6 and 10 mg/L

Measured (mean):	0 (control and solvent control), 0.93, 1.8, 2.4, 4.0 and 6.1 mg/L
Solvent:	Acetone
Test conditions:	
Water quality:	Fortified well water; total hardness 160-170 mg/L as $CaCO_3$
Temperature:	20-21°C (0 - 48 h)
pH:	Range-finding test: not reported Definitive test: 8.00-8.2 (0-48 h)
O_2 content:	Range-finding test: not reported Definitive test: 75-94 % saturation (6.8 – 8.5 mg O_2/L)
Light regime:	16 hours light / 8 hours darkness
Test parameters:	Immobilisation was recorded at 24 and 48 hours after start of exposure. During the exposure the daphnids were not fed. Measurements of pH, temperature and dissolved oxygen concentrations were recorded daily throughout the study. Total hardness, total alkalinity and specific conductance were measured at test initiation in one replicate vessel of each treatment level and control solutions. For chemical analysis of RPA 406341 in the test media samples were taken from both replicates of the high, middle and low treatment levels and the dilution water control, twice prior to the start of the definitive exposure. After start of definitive exposure, samples for analysis were taken at 0 and at 48 hours of exposure.
Statistics:	The EC_{50} value and associated confidence limits at 48 hours were calculated by a computer program (Stephan, 1982, personal communication) with three statistical methods moving average angle analysis, probit analysis and nonlinear interpolation with 95% confidence limits calculated by binomial probability.
Findings:	
Analytical data:	The analytical data of the definitive test resulted in measured concentrations of RPA 406203 ranging from 61 to 81% of the nominal concentrations. Hence, the endpoint is based on mean measured concentrations.
Range finding study:	Following 24 hours of exposure (test termination), undissolved test substance was observed at the bottom of the 10 mg/L vessels. During the same period, immobilization of 10% and 50% was observed among daphnids exposed to the 3.6 and 10 mg/L concentrations, respectively. One of the mobile daphnids exposed to the 10 mg/L concentration was observed to be lethargic and on the bottom of the test vessel.

Table 9.2-24: Effects on daphnids (*D. magna*) exposed to RPA 406203 definitive test

RPA 406203[mg /L] (mean measured)	**Cumulative immobilized organisms [%]**	
	24 hours	**48 hours**
Control	0	0
Solvent control	5	5
0.93	0	0

RPA 406203[mg /L] (mean measured)	Cumulative immobilized organisms [%]	
	24 hours	48 hours
1.8	0	0
2.4	15	20[a]
4.0	20[a]	70[ab]
6.1	55[ab]	95[bc]
48 h EC_{50} = 3.4 mg /L based on mean measured concentration 48 h NOEC = 1.8 mg/L		

[a]Two or more of the mobile daphnids were observed to be lethargic and on the bottom of the test vessel.

[b]Undissolved test substance was observed on the bottom of the test vessel.

[c]All of the mobile daphnids were observed to be lethargic and on the bottom of the test vessel.

Conclusion: The 48-hour EC_{50} was calculated as 3.4 mg/L based on mean measured concentrations.

Comment RMS: The study was evaluated following the recommendations of the currently valid test guidelines OECD 202 (2004)

Check of validity criteria:

- In the control, including the control containing the solubilising agent, not more than 10 per cent of the daphnids should hve been immobilised or show other signs of disease or stress (e.g. discoloration or unusual behaviour). During the test, 5 % immobilisation occurred in the solvent control. Fulfilled.
- The the dissolved oxygen concentration at the end of the test should be ≥ 3 mg/L in control and test vessels. The dissolved oxygen concentration was between 6.8 and 8.5 mg/L during the test. Fulfilled.

In addition, the following points deviated from the test guidelines or were not reported in detail:

- OECD 202 recommends testing of at least 20 daphnids per test group divided into four groups of five animals. In the study, 20 daphnids in divided in two groups of ten animals were tested.
- OECD 202 recommends testing with a reference substance every month and at least twice a year. No information about reference testing is given in the study report.

Acceptability of the analytical methods used in the test: The evaluation of the analytical methods was conducted according to the Guidance Document SANCO/3029/99 rev. 4. Based on the evaluation (see Volume 3 B.5-CA, B.5.1.2.6) the analytical method is considered valid to quantify the amount of triticonazole in fresh and seawater. As RPA 406203 is the isomer to triticonazole the analytical method is acceptable.

LOQ: 0.2493 mg/L

	Endpoints: The RMS recalculated the endpoints by using ToxRat® Pro v. 3.2 providing confidence intervals and agrees on the endpoints given in the study report. EC_{50} = 3.4 mg /L mean measured concentration (95% C.I.: 1.72-2.56 mg/L) NOEC = 1.8 mg/L mean measured concentration **Conclusion of the RMS:** Based on the evaluation of the study the daphnid acute toxicity test is considered valid.

Reference:	**Acute Toxicity of Reg. No. 5079359 (Metabolite of BAS 595 F) to *Daphnia magna* Straus in a 48 hour static test**
Author(s), year:	Janson, G.-M. 2009a
Report/Doc. number:	BASF DocID 2009/1075083
Guideline(s):	OECD 202; OPPTS 850.1010, draft April, 1996
GLP:	Yes
Deviations:	Please refer to the commenting box below
Validity:	Acceptable

Material and methods:

Test substance:	Reg. No. 5079359 (RPA 406203) Purity 99.9.%, batch no.: BESS0578
Test species:	*Daphnia magna*; in house culture
Number of organisms:	4 replicates per treatment, each with 5 daphnids, control and solvent control
Age:	> 2 < 24 hours
Type of test, duration:	static test, 48 hours
Applied concentrations:	
Nominal:	3.5, 4.6, 5.9, 7.7 and 10 mg/L
Measured (mean):	Not given
Solvent:	DMF/Cremophor (v:v, 9:1)
Test conditions:	
Water quality:	Reconstituted water, M4 according to Elendt; total hardness at test initiation 2.4 mmol/L
Temperature:	20.1 - 21°C (0-48 h)
pH:	7.94 - 8.04 (0-48 h)
O_2 content:	8.7 – 9.0 mg O_2/L
Light regime:	16 hours light / 8 hours darkness
Test parameters:	Immobilisation was recorded at 24 and 48 hours after start of exposure. During the exposure the daphnids were not fed. Measurements of pH, temperature and dissolved oxygen concentrations

determined at test start in the new test media (pooled sample) and in one replicate of each concentration at the end of the test (48 h).
For chemical analysis of Reg. No. 5079359 in the test media samples were taken from each test concentration at the beginning and at the end of the test.

Statistics: Statistical calculations were performed with the commercial software ToxRatPro Version 2.10. (ToxRat Solutions GmbH, Alsdorf, Germany).

Findings:

Analytical data: Mean measured concentrations of Reg. No. 5079359 were 98.2 - 105.3% of the nominal concentration at test initiation and 97.6 – 102.4% at test termination.
Hence, the endpoint is based nominal concentrations.

Effects:

Table 9.2-25: Effects on daphnids (*D. magna*) exposed to Reg. No. 5079359

Reg. No. 5079359 [mg /L] (nominal)	Cumulative immobilized organisms [%]	
	24 hours	48 hours
Control	0	0
Solvent control	0	0
3.5	0	0
4.6	0	0
5.9	0	0
7.7	0	0
10	0	15
48 h EC_{50} > 10 mg /L based on nominal concentrations 48 h NOEC = 10 mg/L		

Conclusion: The acute toxicity of Reg. No. 5079359 to *Daphnia magna* has been investigated. The 48-hour EC_{50} was calculated as > 10 mg/L based on nominal concentrations.

Comment RMS: The study was evaluated following the recommendations of the currently valid test guidelines OPPTS 850.1010 (2016) and OECD 202 (2004)

Check of validity criteria:

OPPTS 850.1010 (2016):

- All test vessels are identical. Fulfilled.
- Treatments are randomly or indiscriminately assigned to individual tes vessel locations, individual test organisms were randomly or indiscriminately assigned to test vessels or compartments. Fulfilled.
- A dilution water control (and vehicle (solvent) control, if a vhicle was used) was included in the test. A dilution water control and a solvent control with DMF/Cremophor (v:v, 9:1) were tested in the study. Fulfilled.
- Not more than 10% of the organisms in either the dilution water or vehicle (solvent) controls showed signs of disease, stress (e.g., discoloration, unusual

behavior, immobilization), and/or death. During the test, no immobilisation occurred in the control or solvent control. Fulfilled.
- Daphnids were not fed during the test. Fulfilled.
- No surfactant or dispersant was used in the preparation of a stock or test solution. (However, adjuvants may be used when testing pesticide typical end-use products). Fulfilled.

OECD 202 (2004):
- In the control, including the control containing the solubilising agent, not more than 10 per cent of the daphnids should have been immobilised or show other signs of disease or stress (e.g. discoloration or unusual behaviour). During the test, no immobilisation occurred in the control or solvent control. Fulfilled.
- The the dissolved oxygen concentration at the end of the test should be ≥ 3 mg/L in control and test vessels. The dissolved oxygen concentration was between 8.7 and 9 mg/L during the test. Fulfilled.

Acceptability of the analytical methods used in the test:
The evaluation of the analytical methods was conducted according to the Guidance Document SANCO/3029/99 rev. 4. Based on the evaluation (see Volume 3 B.5-CA, B.5.1.2.6) it could be demonstrated that the method fulfils the requirements with regard to linearity, specificity, accuracy, repeatability, and limit of quantification.
LOQ: 0.001 mg ai/L.
Analyte 5073959 is the cis-isomer of triticonazole and therefore the determination of triticonazole (method APL0500/02) is acceptable. In this adapted method, the LOQ was defined as 1 mg/L.

Endpoints:
The RMS agrees on the EC_{50} given in the study report.
EC_{50} > 10 mg /L nominal concentration
NOEC = 10 mg/L nominal concentration

Conclusion of the RMS: Based on the evaluation of the study the daphnid acute toxicity test is considered valid.

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

Reference:	**An assessment of the effects of RPA 400727 on the reproduction of *Daphnia magna* (Straus)**
Author(s), year:	Douglas, M.T., Halls, R. W. S., Anderson, A. 1992a
Report/Doc. number:	R013032
Guideline(s):	OECD 202 (Part 2, 1984)
GLP:	Yes
Deviations:	Please refer to the commenting box below
Validity:	Not acceptable

Material and methods:

Test substance:	RPA 400727, batch no.: DA640, purity: 95.9%
Test species:	*Daphnia magna* (Straus); laboratory culture originating from a strain supplied by IRChA, France
Number of organisms:	4 replicates each with 10 daphnids per treatment and control group
Age:	< 24 hours old
Type of test, duration:	Semi-static test, Medium renewal 3 times per week (days 2, 4, 7, 9, 11, 14, 16 and 18); 21 days

Applied concentrations:

Nominal:	0 (control and solvent control), 0.1, 0.32, 1.0, 3.2 and 10 mg ai/L
Mean measured	0 (control and solvent control), 0.092, 0.29, 0.93, 2.9, and 9.3 mg ai/L
Solvent:	10% Tween 80-dimethylformamide

Test conditions:

Water quality:	Dechlorinated and aged laboratory tap water, total hardness: 350 mg/L as $CaCO_3$
Temperature:	21 °C
pH:	8.1 – 8.4
O_2 content:	8.3 mg/L (95% air saturation)
Light regime:	16 hours light / 8 hours darkness
Test parameters:	The live and dead Daphnia of the "parental" (P_1) generation were counted daily and recorded together with observations on the general condition and size of the Daphnia as compared with the controls. At each test media renewal the numbers of live and dead "filial" (F_1) Daphnia were recorded. The number of Daphnia with eggs or young in the brood pouch plus the number of discarded unhatched eggs was also determined at this time. Each vessel received approximately 5 ml of a mixed unicellular algal culture supplemented with fry fish food (Liguifry®), daily. Temperature was recorded daily for each flask. Dissolved oxygen, pH and

temperature were measured before and after- each test media renewal.

Analytical measurements: Verification of test concentration was carried out on Days 0 (fresh media), 2, 4, 7, 9, 14, 16, and 21 (old media).

Statistics: EC_{50} values for immobilisation (mortality) of the parental Daphnia were calculated according to the method of Thompson and Weil. EC_{50} values for the effects on reproduction were determined by fitting logistic response curves to the data.

Findings:

Analytical measurements: The analytical data indicated that the mean measured RPA 400727 concentrations were between 86 and 102% of the nominal test concentrations. However, the results are based on mean measured concentrations.

Lethal effects on P_1: Mortality (immobilisation) occurred predominantly during the first week of the study at the two highest test concentrations (2.9 and 9.3 mg ai/L). However, further mortalities (immobilisation) did occur throughout the study indicating prolonged toxicity effects.

Sub-lethal effects on P_1: Statistically significant effects on reproduction were observed at 0.93 mg ai/L and above. Parental Daphnia in the 9.3 and 2.9 mg ai/L groups appeared smaller in size compared with the control groups from the first week of the study and on days 5 and 6 appeared to be slightly paler in colour. On average one unhatched egg per female was produced at the 9.3 mg ai/L treatment level.

Effects on F_1: No toxic effects on the filial generation were apparent.

Table 9.2-26: Effects on daphnids (*Daphnia magna*) exposed to RPA 400727

RPA 40727 [mg ai/L] (mean measured)	% Survival of P_1	No. live young		No. dead young		No. unhatched eggs	
		Total	per female	Total	per female	Total	per female
Control	95	1711	44	0	0	0	0
Solvent control	93	1721	45	0	0	0	0
0.092	93	1669	45	0	0	0	0
0.29	75	1308	39	0	0	0	0
0.93	75	807	23	0	0	0	0
2.9	40	331	19	0	6	<1	0
9.3	20	46	3	0	16	1	0
21 d EC_{50} (P_1 survival) = 2.5 mg ai/L (95% C.I. 1.6 – 4 mg ai/L) 21 d EC_{50} (P_1 reproduction) = 1.3 mg ai/L (95% C.I. 0.88 – 1.9 mg ai/L) 21 d NOEC = 0.092 mg ai/L (survival, reproduction) based on mean measured concentrations							

Conclusion: Long-term exposure of *Daphnia magna* to RPA 400727 resulted in mortality of the immature parental P_1 generation, with further mortalities occurring after sexual

maturation. Impairment of reproduction occurred with the survivors at exposure levels of 0.93 mg ai/L and above. Progressive deterioration in fecundity from day 14 onwards was apparent at the highest treatment level (9.3 mg ai/L). The 21-day NOEC has been determined to be 0.092 mg ai/L.

Comment RMS:	The study was evaluated following the recommendations of the currently valid test guideline OECD 211 (2012). **Check of validity criteria:** - The mortality of the parent animals (female Daphnia) does not exceed 20% in the control(s) at the end of the test. In the test 5% and 7 % mortality were observed in the control and in the solvent control, respectively. Fulfilled. - The mean number of living offspring produced per parent animal surviving at the end of the test in is ≥ 60 in the control(s). The mean number of living offspring per female was 44 and 45 in the control and in the solvent control respectively. Therefore this validity criterion is not met. **In addition, the following points deviated from the test guideline or were not reported in detail:** - OECD 211 recommends 50 -100 mL of medium per vessel for parent animals. In the study the organisms were maintained in 40 mL test solution. - OECD 211 recommends light intensity in a range of 1000-1500 lux. Light intensity is not reported. **Acceptability of the analytical methods used in the test:** Evaluation of the analytical methods (water/sediment/soil) according to the Guidance Document SANCO/3029/99 rev. 4. Method: HPLC Linearity: No information on linearity is given. Accuracy: 2 fortifications levels (each 1 measurements): 0.1 and 10 mg/L; mean recoveries for each level: 99 – 100% Precision: relative standard deviation per fortification level is not given LOQ: not given LOD: = 0.037 mg/L The analytical method fulfils the requirements of guideline SANCO/3029/99 rev. 4. **Endpoints:** EC_{10} and EC_{20} -values were not provided with the study report. 21 d EC_{50} (P1 survival) = 2.5 mg ai/L (95% C.I. 1.6- – 4 mg ai/L), 21 d EC_{50} (P1 reproduction) = 1.3 mg ai/L (95% C.I. 0.88 – 1.9 mg ai/L), 21 d NOEC = 0.092 mg ai/L (survival, reproduction) The endpoints are based on mean measured concentrations.

	Conclusion of the RMS: Based on the evaluation of the study the long-term daphnid toxicity test is not considered valid.

Reference:	**BAS 595 F (Triticonazole) – Full Life-Cycle Toxicity Test with Water Fleas, *Daphnia magna*, Under Static-Renewal Conditions**
Author(s), year:	Putt, E. 2006a
Report/Doc. number:	BASF Reg. Doc. No. 2006/7007209
Guideline(s):	OPPTS Draft Guideline 850.1300
GLP:	Yes
Deviations:	Please refer to the commenting box below
Validity:	Acceptable

Material and methods:

Test substance:	BAS 595 F (Triticonazole), Reg. No. 4378513, purity 90.3%
Test species:	*Daphnia magna*
Number of organisms:	10 replicates each with 1 daphnia per treatment, control and solvent control
Age:	< 24 hours, Springborn Smithers culture
Type of test, duration:	Semi-static test, Medium renewal at test initiation and at 48- or 72-hour intervals
Applied concentrations:	
Nominal:	0 (control and solvent control), 0.19, 0.38, 0.75, 1.5, and 3 mg ai/L
Mean Measured:	0 (control and solvent control), 0.19, 0.37, 0.75, 1.5, and 3 mg ai/L
Solvent:	Dimethylformamide
Test conditions:	
Water quality:	Fortified well water; total hardness: 160 to 170 mg/L as $CaCO_3$
Temperature:	19-21 °C
pH:	7.9 – 8.8
O_2 content:	7.1 – 10.1 mg/L (80 – 105%)
Light regime:	16 hours light / 8 hours darkness; light intensity of 12 to 14 $\mu E \cdot m^{-2} \cdot s-1$, 15 min transition period
Test parameters:	Immobilized adult daphnids and observations of abnormal behaviour were recorded daily. Numbers of offspring were determined upon the first brood release in any vessel, day 8 and daily throughout the remainder test. Additionally the number of immobilized offspring and the time to first brood release were recorded for each treatment level and the controls. At test termination, the total body length and dry weight of the surviving daphnids were measured. During exposure, the food was introduced at a rate of 200 µl of algal suspension (*Ankistrodesmus falcatus*, 4 x 10^7 cells/mL) and 50 µl of yeast, cereal leaves and digested flaked fish food (YCT) suspension, once daily. Dissolved oxygen, pH and temperature were measured before and after each test

media renewal. In addition, the temperature of the water bath was continuously monitored throughout the study. Total hardness, alkalinity and specific conductance were measured and recorded in the freshly prepared solutions of the highest nominal test concentration (3.0 mg ai/L) and the control at test initiation and weekly thereafter.

Analytical measurements: Verification of test concentration was carried out on newly prepared test solutions (days 0, 3, 17 and 19), and from aged test solutions (days 3, 5, 19 and 21).

Statistics: NOEC, LOEC, MATC (Maximum Acceptable Toxicant Concentration) and EC_{50} was estimated. TOXSTAT® version 3.5 (West and Gulley, 1996) was used to perform the statistical computations.

Findings:

Analytical measurements: The analytical data indicated that the mean measured BAS 595 F (triticonazole) concentrations were between 93 and 100% of the nominal test concentrations in new solutions and between 91 and 100% in aged solutions. However, the results are based on mean measured concentrations.

Biological effects: Time of the first brood release in the controls was test day 8 and within normal performance expectation for this species. First brood release in the treatment solutions occurred on day 8 for all treatment levels.

Statistical analysis determined that survival of daphnids was not significantly reduced in any treatment level as compared to the pooled control data. Since ≥ 50% immobilization was not observed in any treatment level during the 21-day exposure, the EC_{50} value for survival was empirically estimated to be > 3 mg ai/L, the highest mean measured concentration tested.

Statistical analysis determined no significant reduction in cumulative offspring per female in any of the treatment levels tested as compared to the pooled control data.

Williams' Test determined a significant difference in total body length among daphnids exposed to all treatment levels tested as compared to the solvent control data. The daphnid length in the four lowest treatment levels exhibited <3% reduction in length compared to the solvent control data

Statistical analysis determined no significant difference in dry body weight in any of the treatment levels tested as compared to the pooled control.

Table 9.2-27: Effects on daphnids (*Daphnia magna*) exposed to triticonazole

Triticonazole [mg ai/L] (mean measured)	Survival of P_1 [%]	No. live young		Mean (SD) total body length [mm]	Mean (SD) dray weight [mg]
		Total	per female (SD)		
Control	90	1183	131 (37)	4.89 (0.05)	0.85 (0.12)
Solvent control	100	1382	138 (26)	4.81 (0.05)	0.83 (0.16)
Pooled control	95	1283	135 (31)	NA	0.84 (0.14)

Triticonazole [mg ai/L] (mean measured)	Survival of P_1 [%]	No. live young		Mean (SD) total body length [mm]	Mean (SD) dray weight [mg]
		Total	per female (SD)		
0.19	100	1127	113 (38)	4.71 (0.11)*	1.05 (0.20)
0.37	100	1370	137 (40)	4.70 (0.08)*	0.96 (0.13)
0.75	100	1212	121 (36)	4.67 (0.17)*	0.99 (0.14)
1.5	90	1100	122 (34)	4.67 (0.10)*	1.03 (0.13)
3	90	1014	113 (27)	4.55 (0.15)*	1.03 (0.16)
21 d EC_{50} (P_1 survival) > 3 mg ai/L 21 d EC_{50} (P_1 reproduction) > 3 mg ai/L 21 d NOEC = 1.5 mg ai/L (length) based on mean measured concentrations					

* Significantly reduced as compared to the solvent control, Williams' test, but not considered biologically significant since percent reductions are < 3% of the solvent control data

** Significantly reduced as compared to the solvent control, based on Williams' Test

SD standard deviation

NA not applicable

Conclusion: Long-term exposure of *Daphnia magna* to Triticonazole resulted in an EC_{50} > 3 mg ai/L and a NOEC of 1.5 mg ai/L based on the argumentation that effects < 3% are not biologically relevant.

Comment RMS: The study was evaluated following the recommendations of the currently valid test guideline OCSPP 850.1300 (2016) and OECD 211 (2012)

Check of validity criteria:

OCSPP 850.1300:

- All test vessels are identical. Fulfilled.
- Treatments are randomly or indiscriminately assigned to individual test vessel locations, individual test organisms were randomly or indiscriminately assigned to test vessels or compartments. Fulfilled.
- A dilution water control (and vehicle (solvent) control, if a vhicle was used) was included in the test. A dilution water control and a solvent control with dimethylformamide were tested in the study. Fulfilled.
- At test begin the daphnids are not more than 24 hours old. Fulfilled.
- Not more than 20% of the organisms in either the dilution water or vehicle (solvent) controls showed signs of disease, stress (e.g., discoloration, unusual behavior, immobilization), and/or death. During the test, 10% immobilisation occurred in the control and no immobilisation the solvent control. Fulfilled.
- Daphnids that live for 21 days in the controls produce on average ≥ 60 offspring

in the 21 days. On average daphnids produced 131 offspring in the control and 138 offspring in the solvent control. Fulfilled.
- No surfactant or dispersant was used in the preparation of a stock or test solution. (However, adjuvants may be used when testing pesticide typical end-use products). Fulfilled.

OECD 211:
- The mortality of the parent animals (female Daphnia) does not exceed 20% in the control(s) at the end of the test. In the test 10% and 0 % mortality were observed in the control and in the solvent control, respectively. Fulfilled.
- The mean number of living offspring produced per parent animal surviving at the end of the test in is ≥ 60 in the control(s). The mean number of living offspring per female was 131 and 138 in the control and in the solvent control respectively. Fulfilled.

In addition, the following points deviated from the test guideline or were not reported in detail:
- OECD 211 recommends that light intensity should not exceed 15-20 µE·m-2·s-1 measured at the water surface of the vessel. Light intensity in the test ranged from 12 to 14 $\mu E \cdot m^{-2} \cdot s-1$.

Acceptability of the analytical methods used in the test: The evaluation of the analytical methods was conducted according to the Guidance Document SANCO/3029/99 rev. 4. Based on the evaluation (see Volume 3 B.5-CA, B.5.1.2.6) the analytical method is considered valid to quantify the amount of triticonazole in fresh and seawater.

LOQ: 0.148 mg ai/L

Endpoints:

In the report a NOEC of 1.5 mg ai/L is chosen based on the argumentation that effects >3% are not biologically relevant. The RMS agrees that such small effects may not be biologically relevant but for precautionary reasons recommends to use the lowest tested concentration as a NOAEC.

21 d EC_{50} (P1 survival) > 3 mg ai/L

21 d EC_{50} (P1 reproduction) > 3 mg ai/L

21 d NOAEC = 0.19 mg ai/L (length)

EC_{10} and EC_{20} -values were not provided with the study report. According to ToxRatPro® v.3.2 EC_{10} and EC_{20} -values could not be calculated.

The endpoints are based on mean measured concentrations.

Conclusion of the RMS: Based on the evaluation of the study the long-term daphnid toxicity test is considered valid.

Reference:	**BAS 595 F (Triticonazole) – Full Life-Cycle Toxicity Test with Water Fleas, *Daphnia magna*, Under Static-Renewal Conditions, Following OPPTS Draft Guideline 850.1300**
Author(s), year:	Urann, K. 2012a
Report/Doc. number:	BASF Reg. Doc. No. 2012/7003660
Guideline(s):	OPPTS Draft Guideline 850.1300
GLP:	Yes
Deviations:	Please refer to the commenting box below
Validity:	Acceptable

Material and methods:	
Test substance:	BAS 595F., batch no.: COD-001440, purity: 91.3%
Test species:	(*Daphnia magna*), Smithers Viscient culture
Number of organisms:	10 replicates each with 1 daphnia per treatment and controls
Age:	< 24 hours
Type of test, duration:	Semi-static test, Medium renewal at test initiation and at 48- or 72- hour intervals thereafter.
Applied concentrations:	
Nominal:	0 (control), 0.1, 0.2, 0.4, 0.8, 1.6 and 3.2 mg ai/L
Mean measured	0 (control), 0.11, 0.21, 0.43, 0.88, 1.8 and 3.5 mg ai/L
Solvent:	Dimethylformamide
Test conditions:	
Water quality:	Fortified well water, total hardness: 180 to 210 mg/L as $CaCO_3$
Temperature:	19-22 °C
pH:	7.7 – 8.6
O_2 content:	7.7 – 10 mg/L (85 - 111% air saturation)
Light regime:	16 hours light / 8 hours darkness, intensity range 71 to 88 footcandles (760 to 950 lux)
Test parameters:	The number of immobilized adult daphnids and observations of abnormal behaviour were recorded daily. Numbers of offspring were determined upon the first brood release in any vessel and daily throughout the remainder the test. In additions, immobilized offspring and the time to first brood release were recorded for each treatment level and the controls. At test termination, the total body length and dry weight of each surviving adult daphnid was measured. During exposure, the food was introduced daily at a rate of 200 µl of algal suspension (*Ankistrodesmus falcatus*, 4 x 10^7 cells/mL) and 50 µl of yeast, cereal leaves and digested flaked fish food (YCT) suspension. This quantity is equivalent to approx. 0.3 mg carbon/daphnid/day. Dissolved oxygen, pH and temperature were measured before and after- each test

media renewal. In addition, the temperature of the water bath was continuously monitored throughout the study. Total hardness, alkalinity and specific conductance were measured and recorded in the freshly prepared solutions of the highest nominal test concentration (3.2 mg ai/L) and the controls at test initiation and weekly thereafter.

Analytical measurements: Verification of test concentration was carried out in newly prepared test solutions on days 0, 2, 16 and 19 and in aged test solutions on days 2, 5, 19 and 21.

Statistics: NOEC, LOEC, MATC (Maximum Acceptable Toxicant Concentration) and EC_{50} was estimated. CETIS™ version 1.8.4.20 (Ives, 2011) was used to perform the statistical computations.

Findings:

Analytical measurements: The analytical data indicated that the mean measured triticonazole concentrations were between 100 and 120 % of the nominal test concentrations. The results are based on mean measured concentrations.

Biological effects: First brood occurred on test day 9 in the 3.2 mg ai/L nominal treatment level and on test day 8 in the remaining treatment levels tested and the controls.

Statistical analysis determined no significant reduction in survival and dry weight among daphnids exposed to any of the treatment levels tested compared to the dilution water control data. Statistical analysis determined a significant reduction in cumulative offspring per female in the treatment levels 0.88, 1.8 and 3.5 mg/L and a significant reduction in total body length in the treatment level 1.8 mg/L. However, regarding total body length no clear dose-response could be determined.

No information is given about the number of unhatched eggs or the number of dead young daphnids.

Table 9.2-28: Effects on daphnids (*Daphnia magna*) exposed to triticonazole

Triticonazole [mg ai/L] (mean measured)	Survival of P_1 [%]	No. live young		Mean (SD) total body length [mm]	Mean (SD) dray weight [mg]
		Total	per female (SD)		
Control	100	1587	159 (14)	4.66 (0.16)	0.76 (0.10)
Solvent control	90	1236	137 (54)	4.68 (0.20)	0.86 (0.14)
0.11	90	1378	153 (11)	4.62 (0.10)	0.73 (0.06)
0.21	100	1413	141 (11)*	4.58 (0.13)	0.71 (0.17)
0.43	100	1431	143 (21)	4.48 (0.31)	0.71 (0.22)
0.88	100	1060	131 (17)*	4.46 (0.40)	0.82 (0.15)
1.8	90	1113	124 (12)*	4.38 (0.21)*	0.90 (0.15)
3.5	100	883	85 (21)*	4.55 (0.07)	0.98 (0.05)
21 d EC_{50} > 3.5 mg ai/L (survival, reproduction, growth) 21 d NOEC = 0.43 mg ai/L					

Triticonazole [mg ai/L] (mean measured)	Survival of P_1 [%]	No. live young		Mean (SD) total body length [mm]	Mean (SD) dray weight [mg]
		Total	per female (SD)		
based on mean measured concentrations					

* Significantly reduced as compared to the dilution water control based on Bonferroni's Adjusted t-Test.
SD standard deviation
NA not applicable

Conclusion: Since no concentration tested resulted in ≥ 50% reduction of survival, reproduction or growth, the 21-day EC_{50} values for *Daphnia magna* survival, reproduction and growth were all empirically estimated to be > 3.5 mg/l, the highest mean measured concentration tested. The 21-day NOEC was determined to be 0.43 mg/L. The 21-day LOEC was determined to be 0.88 mg/L. Based on these results, the MATC value was determined to be 0.62 mg/L.

Comment RMS: The study was evaluated following the recommendations of the currently valid test guideline OCSPP 850.1300 (2016) and OECD 211 (2012)

Check of validity criteria:

OCSPP 850.1300:

- All test vessels are identical. Fulfilled.
- Treatments are randomly or indiscriminately assigned to individual tes vessel locations, individual test organisms were randomly or indiscriminately assigned to test vessels or compartments. Fulfilled.
- A dilution water control (and vehicle (solvent) control, if a vhicle was used) was included in the test. A dilution water control and a solvent control with dimethylformamide were tested in the study. Fulfilled.
- At test begin the daphnids are not more than 24 hours old. Fulfilled.
- Not more than 20% of the organisms in either the dilution water or vehicle (solvent) controls showed signs of disease, stress (e.g., discoloration, unusual behavior, immobilization), and/or death. During the test, 0% immobilisation occurred int the control and 10% immobilisation in the solvent control. Fulfilled.
- Daphnids that live for 21 days in the controls produce on average ≥ 60 offspring in the 21 days. On average daphnides produced 159 offspring in the control and 137 offspring in the solvent control. Fulfilled.
- No surfactant or dispersant was used in the preparation of a stock or test solution. (However, adjuvants may be used when testing pesticide typical end-use products). Fulfilled.

OECD 211:

- The mortality of the parent animals (female Daphnia) does not exceed 20% in the

control(s) at the end of the test. In the test 0% and 10 % mortality were observed in the control and in the solvent control, respectively. Fulfilled.

- The mean number of living offspring produced per parent animal surviving at the end of the test in is ≥ 60 in the control(s). The mean number of living offspring per female was 159 and 137 in the control and in the solvent control respectively. Fulfilled.

In addition, the following points deviated from the test guideline or were not reported in detail:

- According to OECD 211 the temperature of the test media should be within the range 18-22°C. However, for any one test, the temperature should not, if possible, vary by more than 2°C within these limits (e.g. 18-20, 19-21 or 20-22°C) as daily range. In general the temperature ranged between 19 and 21°C throughout the test. However, on days 16 and 20 of the exposure the minimum/maximum thermometer recorded a maximum temperature reading of 22°C. It is not possible to discern from the report if the temperature varied by more than 2 °C on these days.

Acceptability of the analytical methods used in the test: The evaluation of the analytical methods was conducted according to the Guidance Document SANCO/3029/99 rev. 4. Based on the evaluation (see Volume 3 B.5-CA, B.5.1.2.6) the analytical method was found to be satisfactory in terms of specificity, linearity and accuracy for the determination of triticonazole in freshwater.

LOQ: 0.00535 mg/L

Endpoints:

In the report, a NOEC of 0.43 mg/L is suggested based on the argumentation that no clear dose response could be found. The solvent control shows a higher toxicity regarding reproduction than the treatment concentrations with 137 juveniles per female and a standard deviation of 54. In one replicate no reproduction took place at all and it can be assumed that this replicate counts responsible for the high standard deviation in the solvent control.

RMS recalculated the endpoints with ToxRatPro® v.3.2, excluding the outlier from the solvent control. The results show significant reduction of offspring in the treatment levels 0.21, 0.43, 0.88, 1.8 and 3.5 mg/L leading to a NOEC of 0.11 mg/L.

21 d EC_{50} > 3.5 mg ai/L

21 d NOEC = 0.11 mg ai/L

EC_{10} and EC_{20} -values were not provided with the study report. Analysis with ToxRatPro® v. 3.2 indicated that no reliable EC_{10} and EC_{20} values can be calculated.

The endpoints are based on mean measured concentrations.

	Conclusion of the RMS: Based on the evaluation of the study the long-term daphnid toxicity test is considered valid.

Reference:	**Triticonazole – *Daphnia magna* Life-Cycle (21-Day Static Renewal) Chronic Toxicity Study**
Author(s), year:	Mc Elligott, A. 1998a
Report/Doc. number:	R013169
Guideline(s):	EPA/FIFRA Guideline 72-4 (1986)
GLP:	Yes
Deviations:	Please refer to the commenting box below
Validity:	Not acceptable

Material and methods:	
Test substance:	triticonazole, lot no.: 013951, purity: 972 g/kg
Test species:	*Daphnia magna;* laboratory cultures Aquatic Ecotoxicology facilities, Rhône-Poulenc Agro, France
Number of organisms:	Individually held daphnids and daphnids held in groups of five, 22 daphnids per treatment and controls
Age:	< 24 hours
Type of test, duration:	Semi-static test, Medium renewal 3 times per week; 21 days
Applied concentrations:	
Range-finding test	
Nominal:	0 (control), 0.05, 1.2, 0.19, 0.48 and 4 mg ai/L
Definitive test	
Nominal	0 (control and solvent control), 0.08, 0.19, 0.48, 1.2 and 3.0 mg ai/L
Mean measured	0 (control and solvent control), 0.08, 0.19, 0.47, 1.3 and 3.0 mg ai/L
Solvent:	Dimethylformamide
Test conditions:	
Water quality:	Reconstituted water (80% DSW + 20% LC-oligo), total hardness: approx. 155 - 164 mg/L as $CaCO_3$
Temperature:	16.8 (in the solvent control) – 21.1 °C
pH:	7.25 – 8.23
O_2 content:	7.8 – 8.8 mg/L (83 - 101% air saturation)
Light regime:	16 hours light / 8 hours darkness; light intensity 400 – 800 lux (approx. 37-74 footcandles)
Test parameters:	Survival of first generation daphnids, abnormal appearance or behaviour, the number of offspring, the time at which the first off-spring was produced, the presence of any males or winter eggs, the presence of eggs in the brood pouch, the

presence of unhatched eggs of daphnids were reported at test initiation and termination and at days 2, 5, 7, 9, 12, 14, 16 and 19 before transfer of the parent animals to fresh test solutions.

At test termination, the total body length and dry weight were measured and recorded from surviving first generation daphnids.

Feeding three times weekly according to the test renewal schedule with a combination of 0.2 ml of yeast suspension, 0.2 ml of unicellular green algae, 0.8 ml of seaweed extract, Marinure 30 and 0.2 ml of a combination of meat extract and fish food, Tetramin®.

Temperature was recorded daily for each flask. Dissolved oxygen, pH and temperature were measured at each concentration level and control at the initiation (rom bulk preparation) and termination (from one replicate) of each period of exposure. Total hardness and conductivity were measured in new or old solutions from the dilution water control, lowest, medium and highest concentration levels at least once a week.

Analytical measurements: Verification of test concentration was carried out twice during the first week of the test and once weekly thereafter (at the beginning and end of a renewal period).

Statistics: Statistical analysis was performed using SAS programs. Survival of first generation parent daphnids between the exposed groups and the solvent control group was analysed using fisher's Exact Test (1 tail).

A test of detecting outliers was performed using Dixon's test.

The Maximum Acceptable Toxicant concentration (MATC) was determined.

Findings:

Analytical measurements: The analytical data indicated that the mean measured triticonazole concentrations were between 90 and 108% of the nominal test concentrations. The results are based on mean measured concentrations.

Range-finding test: Following 12 days exposure 100 % survival was observed in the control and at the concentrations of 0.05, 1.2 and 2.0 mg/L. A reduction in reproductive output was visually estimated to occur at the concentration of 2.0 mg/L. the concentration of 4.0 mg/L was discontinued following 9 days exposure as no young had been produced, 100% survival was recorded at this time at this concentration.

The first offspring were observed in the control group on day 9 of the test and no ephippia were observed during the test period.

Statistical analysis of parental survival showed no significant reduction in any of the exposed groups compared to the solvent control group. The statistical analysis of the reproductive variable showed a significant reduction in the number of live offspring per female surviving at test termination at the highest tested concentration of 3.0 mg/L. Statistical analysis of the total length data showed that the mean total length of the test organisms was significantly reduced at the highest

tested concentration of 3.0 mg/L. No significant difference occurred between the mean dry weight in the solvent control and any of the test concentrations.

Table 9.2-29: Effects on daphnids (*Daphnia magna*) exposed to triticonazole

Triticonazole [mg ai/L] (mean measured)	Survival of P_1 [%]	No. live young		Mean (SD) total body length [mm]	Mean (SD) dray weight [mg]
		Total	per female (SD)		
Control	57	445	111.3 (12.82)	4.29 (0.05)	0.61 (0.07)
Solvent control	71	647	129.4 (8.2)	4.26 (0.11)	0.60 (0.06)
0.08	71	607	121.4 (23.14)	4.21 (0.11)	0.55 (0.07)
0.19	86	608	120 (6.4)[a]	4.15 (0.19)	0.53 (0.06)
0.47	57	449	112.3 (16.82)	4.18 (0.07)	0.53 (0.02)
1.3	86	752	125.3 (13.78)	4.09 (0.09)	0.55 (0.12)
3.0	43	6	2 (3.46)*	3.93 (0.15)*	0.46 (0.11)
21 d NOEC = 1.3 mg ai/L $MATC_{mean}$ = 1.97 mg/L based on mean measured concentrations					

* Significantly different from the solvent control group ($\alpha = 0.01$).
[a] One daphnid which produced only 8 offspring during the test period was excluded from the statistical analysis of the test data.

Conclusion: Under the conditions of the test and based on the statistical analysis of the test data, the NOEC value of the test substance to *Daphnia magna* was estimated to be 1.3 mg/L and the LOEC was reported to be 3.0 mg/L These estimates were based on a significant reduction in reproductive output and a significant reduction in mean total length of parent daphnids at the mean measured concentration of 3.0 mg/L. The Maximum Acceptable toxicant Concentration (MATC) was established to occur within the range 1.3 and 3.0 mg/L. the geometric mean MATC value was calculated to be 1.97 mg/L.

Comment RMS: The study was evaluated following the recommendations of the currently valid test guideline OCSPP 850.1300 (2016) and OECD 211 (2012)

Check of validity criteria:

OCSPP 850.1300:

- All test vessels are identical. Fulfilled.
- Treatments are randomly or indiscriminately assigned to individual tes vessel locations, individual test organisms were randomly or indiscriminately assigned to test vessels or compartments. Fulfilled.
- A dilution water control (and vehicle (solvent) control, if a vehicle was used) was included in the test. A dilution water control and a solvent control with dimethylformamide were tested in the study. Fulfilled.

- At test begin the daphnids are not more than 24 hours old. Fulfilled.
- Not more than 20% of the organisms in either the dilution water or vehicle (solvent) controls showed signs of disease, stress (e.g., discoloration, unusual behavior, immobilization), and/or death. During the test, 43% immobilisation occurred int the control and 29% immobilisation in the solvent control.
- Daphnids that live for 21 days in the controls produce on average ≥ 60 offspring in the 21 days. On average daphnides produced 111 offspring in the control and 129 offspring in the solvent control. Fulfilled.
- No surfactant or dispersant was used in the preparation of a stock or test solution. (However, adjuvants may be used when testing pesticide typical end-use products). Fulfilled.

OECD 211:
- The mortality of the parent animals (female Daphnia) does not exceed 20% in the control(s) at the end of the test. In the test 43% and 29 % mortality were observed in the control and in the solvent control, respectively.
- The mean number of living offspring produced per parent animal surviving at the end of the test in is ≥ 60 in the control(s). The mean number of living offspring per female was 111 and 129 in the control and in the solvent control respectively. Fulfilled.

In addition, the following points deviated from the test guideline or were not reported in detail:
- According to OCSPP 850.1300 the temperature should be 20°C and should be constant within ± 2 °C. During the test a single temperature measurement from one (old) test solution replicate in the solvent control group was 16.8°C.
- According to OECD 211 the temperature of the test media should be within the range 18-22°C. However, for any one test, the temperature should not, if possible, vary by more than 2°C within these limits (e.g. 18-20, 19-21 or 20-22°C) as daily range. During the test a single temperature measurement from one (old) test solution replicate in the solvent control group was 16.8°C.

Acceptability of the analytical methods used in the test: The evaluation of the analytical methods was conducted according to the Guidance Document SANCO/3029/99 rev. 4. Based on the evaluation (see Volume 3 B.5-CA, B.5.1.2.6) the analytical method was sufficiently validated for determination of triticonazole in freshwater.

LOQ: 50 µg/L

Endpoints:

21 d NOEC = 1.3 mg ai/L

MATCmean = 1.97 mg/L

	Conclusion of the RMS: Based on the evaluation of the study the long-term daphnid toxicity test is not considered valid due to the high mortality observed in the control.

Reference:	**BAS 595 F (Triticonazole) – Life-Cycle Toxicity Test with Mysids (*Americamysis bahia*)**
Author(s), year:	Putt, E. 2006b
Report/Doc. number:	BASF Reg. Doc. No. 2006/7007246
Guideline(s):	OPPTS Draft Guideline 850.1350; FIFRA Guideline 72-4
GLP:	Yes
Deviations:	Please refer to the commenting box below
Validity:	Acceptable

Material and methods:

Test substance:	BAS 595 F (triticonazole), Reg. No. 4378513.: 013951, purity: 90.3%
Test species:	*Americamysis bahia;* Springborn Smithers Laboratories culture
Number of organisms:	Two replicates with two retention chamber, with 15 organisms each; 60 organisms per treatment and controls
Age:	≤ 24 hours
Type of test, duration:	Flow-through; 7.4 aquarium volume additions per day, 28 days
loading	0.018 g of biomass per liter of flowing test solution per day
Applied concentrations:	
Range-finding test	
Nominal:	0 (control and solvent control with dimethylformamide), 0.063, 0.13, 0.25, 0.5 and 1 mg ai/L
Definitive test	
Nominal	0 (control and solvent control), 0.019, 0,038, 0,075, 0.15 and 0.3 mg ai/L
Mean measured	0 (control and solvent control), 0.025, 0.041, 0.085, 0.16 and 0.32 mg ai/L
Solvent:	Triethylene glycol (TEG)
Test conditions:	
Water quality:	Artificial seawater formulated by addition of commercial salt mix to laboratory well water, total hardness: approx. 152 - 170 mg/L as $CaCO_3$ in newly prepared batches, TOC concentration 0.35 mg/L
Temperature:	25-27 °C
pH:	8.1 – 8.4
O_2 content:	4.5 – 7.3 mg/L (60% at 4.4 mg/L at 20‰ and 25 °C)
Salinity	19-21‰
Light regime:	16 hours light / 8 hours darkness; light intensity 260 – 620 lux (approx. 24-58

	footcandles)
Test parameters:	Survival of male, female and male/female combined mysids at test termination, growth (average dry body weight and average total body length) of both male and female mysids and reproduction (the number of young released per female). Reproduction was determined only for the paired organisms. Feeding twice daily with brine shrimp (*Artemia salina*) nauplii, partly enriched with Selco®. Dissolved oxygen, pH, temperature and salinity were measured daily in each replicate of each treatment level and the controls. In addition, exposure solution temperature was continuously monitored in one control vessel.
Analytical measurements:	Concentration prior to the start of the definitive exposure was analysed in the dilution water control and the high, middle, and low test concentrations. Verification of test concentration was carried on test days 0, 6, 12, 20 and 28 in alternate replicate test solutions of each treatment level and control.
Statistics:	Statistical analysis was performed using TOXSTAT® Version 3.5 (West, Inc. and gulley, 1996). Determination of NOEC, LOEC and MATC is based on the most sensitive of the performance criteria evaluated.

Findings:

Analytical measurements: The analytical data indicated that the mean measured triticonazole concentrations were between 100 and 130% of the nominal test concentrations. The results are based on mean measured concentrations.

Table 9.2-30: Effects on mysids (*Americamysis bahia*) exposed to triticonazole, range-finding test

Triticonazole [mg ai/L] nominal	**Mean survival**	**Mean number offspring per female**	**Mean total length [mm]**		**Mean dry weight [mg]**	
			males	**females**	**males**	**females**
Control	75	20.1	7.7	7.9	1.0	1.24
Solvent control	86	3.2	7.1	7.6	0.88	1.12
Pooled control	81	11.6	NA	7.8	0.94	1.18
0.063	89	2.6	7.1	7.3	0.86	1
0.13	80	1.5	7.2	7.2*	0.9	0.97*
0.25	87	2.0	7.1	7.3	0.83	1.02
0.5	84	1.1	6.8	7.2*	0.75	0.96*
1.0	88	0.9	6.8	6.9*	0.66	0.86*

* Statistically reduced compared to the pooled control data
NA not applicable

Table 9.2-31: Effects on mysids (*Amerciamysis bahia*) exposed to triticonazole, definitive test

Triticonazole [mg ai/L] Mean measured	Mean survival F_0			Mean number offspring per female	Mean total length [mm]		Mean dry weight [mg]	
	male	female	male/ female		males	females	males	females
Control	83	100	94	6.8	7.4	7.7	0.95	1.21
Solvent control	80	100	92	5.2	7.3	7.5	0.90	1.16
Pooled control	82	100	93	6.0	7.4	7.6	0.93	1.19
0.025	100	96	98	4.7	7.5	7.7	0.98	1.24
0.041	90	89	89	4.0	7.4	7.6	0.94	1.24
0.085	86	90	90	2.2*	7.3	7.5	0.84	1.13
0.16	90	88	89	1.8*	7.3	7.5	0.88	1.09
0.32	75	96	85*	1.4*	7.2	7.4	0.81*	1.08
LC_{50} > 0.32 mg ai/L NOEC = 0.041 mg ai/L (reproduction) $MATC_{mean}$ = 0.059 mg ai/L								

* Significantly different compared to the pooled control, based on William' Test
NA not applicable

Conclusion: Based on statistical analysis of mysid reproduction (determined to be the most sensitive indicator of toxicity), a LOEC of 0.085 mg ai/L, a NOEC of 0.041 mg ai/L and a $MATC_{mean}$ of 0.059 mg ai/L was determined. Since no concentration resulted in ≥ 50% reduction in survival, the 28-day LC_{50} value was empirically estimated to be > 0.32 mg ai/L.

Comment RMS: The study was evaluated following the recommendations of the currently valid test guideline OPPTS 850.1350 (1996) draft

Check of validity criteria:

OPPTS 850.1350 draft:

- Not more than 25% of first generation females in the control groups fail to produce young. During the test 100% of the females in the control and 95% of the females in the solvent control produced young. Fulfilled.
- Average number of young produced per female in the controls is > 3 per day. During the test in the control 6.8 and in the solvent control 5.2 young were produced per female. Fulfilled.

In addition, the following points deviated from the test guideline or were not reported in detail:

- According to OPPTS 850.1350 draft the measurement of length should be

	conducted with living organisms. In the study the length was measured after termination. - According to OPPTS 850.1350 draft the measured concentration of the test substance should not vary more than 20 percent among replicate test chambers of a treatment concentration. In the study the mean measured triticonazole concentrations were between 100 and 130% of the nominal test concentrations. - OPPTS 850.1350 draft recommends a 14-hour light and 10-hour dark photoperiod with a 15- to 30 min transition period. During the test 16 hours light and 8 hours dark were provided. - OPPTS 850.1350 draft body length should be measured at the first observation day and on day 28. In the study body length was only measured at test termination. **Acceptability of the analytical methods used in the test:** The evaluation of the analytical methods was conducted according to the Guidance Document SANCO/3029/99 rev. 4. Based on the evaluation (see Volume 3 B.5-CA, B.5.1.2.6) the analytical method is considered valid to quantify the amount of triticonazole in fresh and seawater. LOQ: 0.45 µg ai/L **Endpoints:** The RMS agrees on the endpoints given in the study report LC_{50} > 0.32 mg ai/L NOEC = 0.041 mg ai/L (reproduction) $MATC_{mean}$ = 0.059 mg ai/L EC_{10} and EC_{20} -values were not provided with the study report. The endpoints are based on mean measured concentrations. **Conclusion of the RMS:** Based on the evaluation of the study the long-term mysid toxicity test is considered valid.

Reference:	**Triticonazole: chronic effects on midge larvae (*Chironomus riparius)* in a water/sediment system**
Author(s), year:	Van der Kolk, J., 1998
Report/Doc. number:	R005755
Guideline(s):	BBA guideline (1995)
GLP:	Yes
Deviations:	Please refer to the commenting box below
Validity:	Borderline

Material and methods:	
Test substance:	Triticonazole, batch no.: 9802018, purity: 965 g ai/kg
Test species:	Midge (*Chironomus riparius*), cultured in Srpingborn Laboratories (Europa) AG, original source University of Sheffield 1991
Number of organisms:	4 replicates, each with 25 larvae per treatment and control
Age:	First instar larvae, approx. 1-2 day old
Type of test, duration:	Static test, 26 days
Feeding:	Ziegler Brother Prime Flakes suspension (50 mg/ml), 0.5 – 1 ml per test vessel on most days during first half of the test; 0.25 ml on day 17 during the second half of the test
Applied concentrations:	
Nominal (overlying water):	0 (control), 6.25, 12.5, 25, 50 and 100 µg ai/L
Mean measured:	0 (control), 2.11, 4.33, 9.03, 23.36,61.39 µg ai/L
Solvent:	None
Test conditions:	
Water quality:	Elendt M4 Medium (deionized, reconstituted town of Horn well water)
Temperature:	19.9 – 22.2 °C
pH:	6.36 – 8.67
Hardness:	234 mg/L as $CaCO_3$
O_2 content:	4.67 – 11.17 mg O_2/L (53 - 125% air saturation)
Light regime:	16 hours light / 8 hours darkness
Test sediment:	Artificial sediment according OECD guideline 207. Mixture of 10% sphagnum peat, 20% kaolin clay, 70% industrial sand (pH: 5.85)
Test system:	A layer of approx. 2 cm of sediment (210 ml) and 16.5 cm (1750 ml) of overlying water in 2-liter glass beakers. Larvae of the first larval stage were added impartially to the test system.
Test parameter:	The test vessels were inspected daily for emerged midges. The number of male and female midges emerged and the number of pupae not emerged were recorded. The temperature, pH and dissolved oxygen were recorded once a week in all test

vessels. The temperature of the room in which the test was performed was continuously monitored.

Each day the aeration of the test vessels was checked and adjusted if necessary.

Analytical measurements: Samples of the overlying water were taken 1 hour, 7 days and 26 days after application. The samples for each dose group were combined for analysis.

Statistics: The NOEC and LOEC for emergence rate and development rate were calculated using a multiple t-test (Williams test) at p = 0.05, one sided. (Williams 1971 and 1972).

Findings:

Analytical measurements: At hour 0, recoveries of 48.0 and 77.7% were found. Part of the test item had fallen out of the test solution. The recoveries of triticonazole decreased to 27.5 – 63.9% on day 7 and to 27.8 – 46.4% on day 26.

As the last adult emergence was observed on day 20 in the control group, the test was stopped at day 26 (more than 5 days after the last emergence).

Table 9.2-32: Emergence rate and development rate of *Chironomus riparius*

Triticonazole [µg ai/L] (nominal)	Emergence rate Mean ± std.	Development rate Mean ± std.
Control	0.93 ± 0.07	0.072 ± 0.001
6.25	0.90 ± 0.12	0.071 ± 0.002
12.5	0.93 ± 0.08	0.073 ± 0.001
25	0.91 ± 0.05	0.068 ± 0.001*
50	0.95 ± 0.04	0.070 ± 0.003
100	0.96± 0.05	0.070 ± 0.003

* Significantly lower compared to the control

Conclusion: Triticonazole, applied at a mean measured concentration of 100 µg ai/L to a sediment-water system had no significant effect on emergence rate and development rate of *Chironomus riparius*. Hence, a NOEC of 100 µg ai/L (was determined. The EC_{50} (emergence, development) was determined to be greater than 61.39 µg/L.

Comment RMS:	The study was evaluated following the recommendations of the currently valid test guideline OECD 219 (2004) **Check of validity criteria:** - The emergence in the controls must be at least 70% at the end of the test. At the end of the study the emergence in the control vessels ranged between 84 and 100%. Fulfilled. - *C riparius* emergence to adults from control vessels should occur between 12 and 23 days after their insertion into the vessels. During the test, emergence to adults occurred between day 12 and day 20. Fulfilled.

- At the end of the test, pH and the dissolved oxygen concentration should be measured in each vessel. The oxygen concentration should be at least 60 per cent of the air saturation value at the temperature used, and the pH of overlying water should be in the 6-9 range in all test vessels. During the test, the pH varied between 6.36 and 8.67, the dissolved oxygen concentration varied between 53 and 125% of air saturation, whereas it is not reported at which points of time and how long it decreased lower 60%.
- The water temperature should not differ by more than ± 1.0°C. The water temperature could be controlled by isothermal room and in that case the room temperature should be confirmed in appropriate time intervals. During the test, the temperature ranged between 19.9 and 22.2°C. According to the continuous temperature monitoring of the test room the temperature remained at 20 ± 2 °C.

In addition, the following points deviated from the test guideline or were not reported in detail:

- According to OECD 219 quantification of the test substance in overlying water, pore water and sediment with known and reported accuracy and limit of detections should be available. In the study only the quantification of the test substance in overlying water was conducted.
- According to OECD 219 the sediment/water ratio should not exceed 1:4. The ratio in the test was 1:8.
- According to OECD 219 a reference substance should be tested periodically. The study report contains no information regarding a reference test.
- According to OECD 219 light intensity should be 500 to 1000 lux. The light intensity was not reported in the test.

Acceptability of the analytical methods used in the test: Evaluation of the analytical methods (water/sediment/soil) according to the Guidance Document SANCO/3029/99 rev. 4.

Method: HPLC

Linearity: Calibration is based on duplicate determination at five concentrations.

The calibration function was linear within the range from 19.57 – 148.7 with r2 = 0.9976

Accuracy: 3 fortifications levels (each 2 measurements): 4.864, 24.32 and 58.35 LOQ. Mean recoveries for each level: 88.94-110%

Precision: The relative standard deviation per fortification level ≤ 20 %

LOQ: 0.9029 µg/L

LOD: not given

The analytical method fulfils the requirements of guideline SANCO/3029/99 rev. 4.

Endpoints:

In the study report a NOEC of 100 µg ai/L and an EC_{50} > 100 µg ai/L, based on nominal concentrations is stated. However, the endpoints should be based on initially measured concentrations according to the OECD test guideline. Furthermore the concentrations decreased to 27.8-46.4% of nominal concentration at the end of the test. Therefore the endpoints are as follows:

EC_{50} > 77.7 µg ai/L

NOEC = 77.7 µg ai/L

The endpoints are based on initial mean measured concentrations

EC_{10} and EC_{20} -values were not provided with the study report.

However as in the study only the quantification of the test substance in overlying water was conducted, the accuracy of the exposure is not known.

Conclusion of the RMS: Based on the evaluation the reliability of the long-term *Chironomus riparius* toxicity test is questionable as the accuracy of the exposure is not known. Furthermore a sediment endpoint would be relevant for the risk assessment as triticonazole is known to highly accumulate in the sediment.

The RMS would highly appreciate opinions from the member state regarding the reliability and of this study and usefulness of the endpoint for the risk assessment.

The notifier provided a statement supporting the validity and reliability of the study. The statement is presented in *italic* below:

Notifier response to AGES's questions regarding aquatic studies with triticonazole

Author: Dr. Philipp Janz; 14th September 2017

BASF believes that the Chironomus study is valid and reliable and can therefore be used in the aquatic risk assessment for triticonazole. In addition, studies on sediment dwelling organisms are not triggered for triticonazole in accordance with the EFSA Aquatic Guidance Document (EFSA AGD, 2013). In conclusion, even if the sediment-water Chironomus study was invalidated (which is not justified in BASF's point of view) this would not lead to a data gap in the aquatic risk assessment for triticonazole.

<u>Validity and reliability of the study</u>

Indeed, the oxygen saturation of the water was below 60% in some cases in the Chironomus study. However, the percentages of oxygen saturation (≥53%) were only slightly below the recommended minimum of 60% in nine out of 24 replicates and the reduced oxygen levels occurred only at the beginning of the study (day 0) (Table 9.2-33). From the second measurement (day 5) on the dissolved oxygen levels ≥71% were clearly above the recommended minimum in all replicates until test termination. It should be noted that, according to OECD TG 219 (2004), the validity criterion for oxygen saturation is not a strict criterion but rather a recommendation and refers to the end of the test (and not to the beginning). In the corresponding section of the OECD TG 219 on the validity criteria it is stated that 'at the end of the test (…) the oxygen concentration should be at least 60 per cent

of the air saturation value (ASV) at the temperature used'. At the end of the test the ASV were in a range of 100-111% in the Chironomus study. Furthermore, the essential validity criteria concerning the development of test organisms were evidently met. The emergence rate in the controls (93%) was above the required 70% and emergence to adults from control vessels occurred between 12 and 23 days after insertion into the vessels as specified in the OCED TG 219. The compliance with the validity criteria for the biological parameters shows that there was no negative impact on the test organisms due to the slightly lower oxygen levels at test initiation. Thus, the results obtained in the Chironomus study are considered reliable without restrictions.

Table 9.2-33: Raw data on dissolved oxygen (%) in water from the sediment-water study with Chironomus riparius (R005755 / BASF DocID 1998/7002186).

Dissolved Oxygen (%)

Dose group µg/l	Replicate	Day 0	Day 5	Day 13	Day 21	Day	mean	std.	min	max
Control	A	61%	87%	82%	114%	104%	90%	20%	61%	114%
	B	60%	76%	83%	119%	107%	89%	24%	60%	119%
	C	60%	79%	77%	103%	103%	84%	18%	60%	103%
	D	58%	78%	85%	119%	104%	89%	23%	58%	119%
6.25	A	59%	78%	92%	117%	106%	91%	23%	59%	117%
	B	60%	87%	87%	118%	103%	91%	21%	60%	118%
	C	57%	79%	79%	115%	104%	87%	23%	57%	115%
	D	61%	86%	88%	111%	105%	90%	20%	61%	111%
12.5	A	57%	71%	74%	114%	108%	85%	25%	57%	114%
	B	53%	85%	81%	116%	103%	88%	24%	53%	116%
	C	62%	85%	86%	115%	102%	90%	20%	62%	115%
	D	61%	86%	74%	111%	100%	87%	20%	61%	111%
25	A	60%	80%	87%	117%	102%	89%	21%	60%	117%
	B	61%	75%	86%	117%	101%	88%	22%	61%	117%
	C	60%	80%	88%	116%	100%	89%	21%	60%	116%
	D	60%	77%	78%	113%	101%	86%	21%	60%	113%
50	A	59%	78%	75%	113%	101%	85%	22%	59%	113%
	B	58%	75%	81%	117%	103%	87%	23%	58%	117%
	C	62%	82%	73%	114%	103%	87%	22%	62%	114%
	D	62%	86%	86%	120%	105%	92%	22%	62%	120%
100	A	60%	78%	78%	115%	107%	88%	23%	60%	115%
	B	59%	78%	86%	125%	113%	92%	27%	59%	125%
	C	59%	78%	88%	110%	111%	89%	22%	59%	111%
	D	61%	84%	82%	119%	109%	91%	23%	61%	119%

For the Chironomus study with triticonazole spiked water was selected as exposure scenario to simulate the route of entry of triticonazole into the water column. The test concentrations of triticonazole were measured in water but not in pore-water or sediment. The analytical verification of test concentrations in water is deemed sufficient because it is state-of-the-art to compare endpoints obtained in water-sediment studies based on concentrations in water (µg/L) with PECSW values for the water phase (µg/L) in the risk assessment for plant protection products (see EFSA AGD).

Besides, the OECD 219 says that 'measurements in sediment might not be necessary if the partitioning of the test substance between water and sediment has been clearly determined in a water/sediment study under comparable conditions' (page 8). In case of triticonazole such a water-sediment study is available and, thus, the measurement of triticonazole in sediment (and pore-water) is not mandatory in a spiked-water toxicity study with Chironomus.

In summary, since the study on Chironomus is valid and provides a reliable endpoint based

on test concentrations in the most relevant environmental matrix (i.e. the concentration in water), it is considered suitable for regulatory risk assessment purposes.

Is triticonazole triggered into testing with sediment dwelling organisms?

The EFSA AGD (2013) defines two conditions under which toxicity studies with sediment-dwelling organisms need to be conducted. The two conditions relate to the environmental fate and the aquatic toxicity of the active substance. When accumulation of the active substance in aquatic sediment is indicated by environmental fate studies (i.e. water-sediment study showed >10% of applied radioactivity at or after day 14 present in the sediment) and the chronic daphnia test (or other comparable study with insects) results in EC10 (or NOEC) <0.1 mg/L), toxicity studies with sediment dwelling organisms are required (see footnotes on page 75 and 79 in the EFSA AGD). Because the chronic Daphnia NOEC for triticonazole is 0.43 mg/L and comparable studies with insects are not available the second condition is not fulfilled for triticonazole. Thus, toxicity studies with sediment dwelling organisms are not required for triticonazole in accordance with the EFSA AGD (2013). Consequently, even if no valid study with sediment dwelling organisms was available for triticonazole there would be no data gap.

Comment RMS:

Validity and reliability of the study

The raw data of the oxygen content show that the study can be considered valid regarding this parameter provided that the column called "Day" presents the measurement at the end of the test on day 26.

Regarding the quantification of the test substance in pore water and sediment the Notifier argues that a water-sediment study under comparable conditions is available determining the partitioning of the test substance between water and sediment. However, upon consultation with the environmental fate expert, the water/sediment studies were not conducted under comparable conditions as for example the sediment to water ratio was not the same.

Is triticonazole triggered into testing with sediment dwelling organisms?

The endpoint of 0.43 mg ai/L proposed in the study is not considered to be the most sensitive endpoint for *Daphnia* long- term toxicity as the RMS proposes 0.11 mg ai/L as valid endpoint of the study.

In the data requirements it is stated that the impact on a sediment-dwelling organism shall be assessed, if accumulation of an active substance in aquatic sediment is indicated or predicted by environmental fate studies.

The aquatic guidance recommends as trigger for sediment dwelling organism testing a daphnid long-term toxicity of < 0.1 mg ai/L for daphnid long-term toxicity in combination with >10% of applied radioactivity at or after day 14 present in the sediment.

The water/sediment study shows that triticonazole partitioned from water to sediment by more

	than 50% after 14 days and more than 70% after 105 days. Triticonazole therefore highly accumulates in the sediment. The available endpoints for daphnids of 0.11 mg ai/L and 0.19 mg ai/L are above the trigger but only very marginal. Therefore the RMS considers a study on sediment dwelling organisms necessary.

B.9.2.7. Effects on algal growth

Laboratory studies on the toxicity to *Pseudokirchneriella subcapitata* (formerly known as *Selenastrum capricornutum*) and the blue green algae *Anabaena flos-aquae* with the active substance triticonazole were submitted for the first EU approval of the active substance. For the current approval laboratory studies with the green algae *Pseudokirchneriella subcapitata*, the diatom *Navicula Pelliculosa*, and the saltwater diatom *Skeletonema costatum* were submitted.

In addition, studies with the metabolite RPA 406203 and the intermediate [redacted] were conducted.

The study summaries are given below.

Active substance:

Reference:	**Assessment of the algistatic effect of RPA 400727 to *Selenastrum capricornutum***
Author(s), year:	Handley, J.W., Mead, C., Bartlett, A.J., 1992a
Report/Doc. number:	R013056
Guideline(s):	OECD test guideline 201, US EPA Pesticide Assessment Guidelines Sub-Division E, Section 72-2
GLP:	Yes
Deviations:	Please refer to the commenting box below
Validity:	Not acceptable

Material and methods:

Test substance:	RPA 400727., lot no.: DA646, purity: 96.8%
Test species:	Green alga (*Pseudokirchneriella subcapitata* formerly known as *Selenastrum capricornutum)*
Number of organisms:	1.22 x 10^4 cells/mL (control), 1.56 x 104 cells/mL (solvent control); Limit test with 6 replicates for treatment group and 3 replicates for each for the control groups
Type of test, duration:	Static test, 96 hours
Applied concentrations:	
Range-finding test	
Nominal:	0.01, 0.1 and 1 mg ai/L
Limit test:	
Nominal:	0 (control and solvent control) and 1 mg ai/L
Solvent:	Tetrahydrofuran
Test conditions:	
Water quality:	Algal culture medium

Temperature:	24 °C
pH:	7.5 – 7.8 (0 h), 9.4 – 9.6 (69 h)
Initiation:	Continuous illumination at ~ 7000 lux
Analytical measurements:	Verification of test concentration was conducted at 0 and 96 hours.
Test parameters:	Samples were taken at 0, 24, 48, and 72 and 96 hours and the absorbance measured at 665 nm. The cell densities of the control cultures at initiation and at termination were determined by direct counting with the aid of a haemacytometer. pH was measured at 0 and 96 hours.
Findings:	
Analytical data:	Mean measured concentrations were in the range of 90.2 – 101.1% of nominal concentrations over the whole test duration. Hence, the results are based on nominal concentrations.
Morphological effects:	After 96 h of exposure no abnormalities were observed in any of the control or treatment groups. The cell concentration of the control and solvent control culture increased by a factor of 26 and 19, respectively.

Table 9.2-34: Effects of RPA 400727the green algae *Selenastrum capricornutum*

RPA 400727 [mg/L] (nominal)	Biomass		Growth rate	
	Area under the curve (96 h)	% inhibition	0 – 24 h	% inhibition relative to the control
Solvent control	23.03	-	0.060	-
1	23.27	<1>	0.058	4

Conclusion:

96 h E_bC_{50} > 1.0 mg ai/L

24 h E_rC_{50} > 1.0 mg ai/L

72 h NOEC = 1.0 mg ai/L

based on nominal concentrations

Comment RMS:

The study was evaluated following the recommendations of the currently valid test guideline OECD 201 (2006)

Check of validity criteria:

- The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period. In the test the cell concentration of the control and solvent control culture increased by a factor of 26 and 19, respectively, after a 96 hour period. Fulfilled.
- The mean coefficient of variation for section-by-section specific growth rates (day 0-1, 1-2 and 2-3, for 72-hour tests) in the control cultures must not exceed 35%. No coefficient of variation is derived in the study and no data available to do so.

- The coefficient of variation of average specific growth rates during the whole test period in replicate control cultures must not exceed 7% in tests with *Pseudokirchneriella subcapitata*. No coefficient of variation is derived in the study and no data available to do so.

In addition, the following points deviated from the test guideline or were not reported in detail:

- According to OECD 201 reference substances should be tested at least twice a year. No information is given in the study report about reference testing.
- According to OECD 201 the cultures should be maintained at a temperature in the range of 21 to 24 °C, controlled at ± 2 °C. In the study report no detailed information is given about the temperature measurement (how it was measured and how often it was measured).
- According to OECD 201 the pH of the control medium should not increase by more than 1.5 units during the test. During this study the pH of the control and solvent control varied between 7.8 and 9.6 and between 7.6 and 9.6, respectively.

Acceptability of the analytical methods used in the test: Evaluation of the analytical methods (water/sediment/soil) according to the Guidance Document SANCO/3029/99 rev. 4.

Method: HPLC

Linearity: The study report states that a range of standard solutions were prepared and the concentration ratio was determined to be linear over 0 to 150%. However, no values are given. r^2 not given.

Accuracy: 2 fortifications levels (each 1 measurement): The measurement does not include 1 x LOQ (0.02 mg/L) Mean recoveries for each level: 94.7 – 100.3%

Precision: not given

LOQ: 0.02 mg/L

LOD: = 0.004 mg/L

The analytical method fulfils the requirements of guideline SANCO/3029/99 rev. 4.

Endpoints:

96 h E_bC_{50} > 1.0 mg ai/L

24 h E_rC_{50} > 1.0 mg ai/L

72 h NOEC = 1.0 mg ai/L

based on nominal concentrations

Conclusion of the RMS: Based on the evaluation of the study the long-term *Selenastrum capricornutum* toxicity test is not considered valid as insufficient information is given in the study report and the raw data has not been enclosed.

Reference:	**Triticonazole-Toxicity to the freshwater blue-green alga, *Anabaena flos-aquae***
Author(s), year:	Hoberg, J.R., 1998a
Report/Doc. number:	R012015
Guideline(s):	FIFRA Guideline 122-2
GLP:	Yes
Deviations:	Please refer to the commenting box below
Validity:	Not acceptable

Material and methods:

Test substance:	Triticonazole tech., batch no. : OP9750057, purity : 90.52%
Test species:	*Anabaena flos-aquae*, Springborn (Wareham) culture
Number of organisms:	1 x 10^4 cells/mL; 3 replicates each, per treatment group and control groups
Type of test, duration:	Static test, 5 days
Applied concentrations:	
Range-finding test	
Nominal:	0.00099, 0.0099, 0.099, 0.99, 9.9 mg ai/L
Definitive test	
Nominal:	0 (control and solvent control), 3.0 mg ai/L
Mean measured:	0 (control and solvent control), 2.6 mg ai/L
Toxic reference	none
Solvent:	Dimethylformamide
Test conditions:	
Water quality:	Algal Assay Procedure medium prepared with sterile, deionized water
Temperature:	25 °C
pH:	7.5 (0 h), 8.2– 8.3 (5 d)
Incubation:	Continuous illumination, 3100 to 3200 lux
Test parameters:	Daily cell count via haemacytometer and a compound microscope and observation of the health of the algal cells. Temperature was measured daily and additionally continuous in a flask of water adjacent to the test flasks. Light intensity was measured at 0 hour and at each 24-hour interval during the exposure period. pH and conductivity were measured at test initiation and at test termination. For chemical analysis of the test substance, samples of test solution were taken at test initiation and at test termination.
Statistics:	A t-Test (Sokal and Rohlf, 1981) was conducted to statistically compare the treatment level cell density with control and solvent control. EC_{25} and EC_{50} were not calculated, but empirically estimated.

Findings:

Range- finding test	At test termination, cell densities in the 0.00099, 0.0099, 0.099, 0.99, 9.9 mg ai/L

treatment levels averaged 217, 214, 207, 204 and 22 x 10^4 cells/mL.

Analytical data: The measured test concentrations were in the range of 95.3 and 103% of nominal concentrations.

Table B. 9.2.7-1: Effects of technical triticonazole on the blue-green algae *Anabaena flos-aquae*

Triticonazole [mg/L] (mean measured)	Cell density [x 10^4 cells/mL)] (SD)					
	Observation interval					% inhibition relative to the pooled control
	Day 1	Day2	Day 3	Day 4	Day 5	
Control	5.3 (1.5)	11 (2.2)	29 (2.1)	88 (5.4)	153 (7.3)	NA
Solvent control	6.0 (1.8)	13 (1.8)	28 (2.3)	89 (3.4)	153 (5.2)	NA
Pooled control	NA	NA	NA	NA	153 (5.7)	NA
2.6	2.9 (1.0)	8.0 (1.4)	21 (2.8)	80 (3.5)	123 (4.2)*	20

SD standard deviation
NA not applicable
*significantly reduced compared to pooled control, based on t-Test

Conclusion: 96 h EC_{50} > 2.6 mg ai/L
NOEC < 2.6 mg ai/L
based on mean measured concentration.

Comment RMS: The study was evaluated following the recommendations of the currently valid test guideline OECD 201 (2006)

Check of validity criteria:

- The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period. In the test the cell concentration of the control and solvent control culture increased by a factor of 16.6 and 14.8, respectively, after a 96 h period. The solvent control does not fulfill the variability criteria, however, at test termination after 120 hours the factor was 25. 5.
- The mean coefficient of variation for section-by-section specific growth rates (day 0-1, 1-2 and 2-3, for 72-hour tests) in the control cultures must not exceed 35%. No coefficient of variation was derived in the study.
- The coefficient of variation of average specific growth rates during the whole test period in replicate control cultures must not exceed 10% in tests with *Anabaena flos-aquae*. No coefficient of variation is derived in the study.

In addition, the following points deviated from the test guideline or were not reported in detail:

- According to OECD 201 reference substances should be tested at least twice a year. No information is given in the study report about reference testing.
- According to OECD 201 a limit test can be conducted at 100 mg/L or a concentration equal to the limit of solubility. The limit of solubility in the test was 10 g/L, the limit test was conducted only with nominal 3 mg/L. Furthermore the

	Guideline requires at least six treatment replicates. In this study only three replicates were tested **Acceptability of the analytical methods used in the test:** Evaluation of the analytical methods (water/sediment/soil) according to the Guidance Document SANCO/3029/99 rev. 4. Method: HPLC Linearity: Calibration is based on duplicate determination at five concentrations. The calibration function was linear within the range from 0.5 – 7.00 mg/L with r^2 = 0.993240. Accuracy: 3 fortifications levels (each 3 measurements): 15.0, 7.00, 0.500 mg/L; Mean recoveries for each level: 70.8-106% Precision: The relative standard deviation per fortification level ≤ 20 % LOQ: 0.2493 mg/L. LOD: not given The analytical method fulfils the requirements of guideline SANCO/3029/99 rev. 4. **Endpoints:** No E_rC_{50} or E_bC_{50} was reported. The Cell density inhibition at termination of the test was 20% based on the mean measured concentration of 2.6 mg/L. **Conclusion of the RMS:** Based on the evaluation of the study the long-term *Anabaena flos-aquae* toxicity test is not considered valid as insufficient information is given in the study report furthermore the limit test was conducted with a lower concentration than recommended in the guidelines and with only three treatment replicates instead of at least six.

Reference:	**Triticonazole – toxicity to the freshwater green alga, *Selenastrum capricornutum***
Author(s), year:	Hoberg, J.R., 1998c
Report/Doc. number:	R012017
Guideline(s):	FIFRA Guideline 122-2
GLP:	Yes
Deviations:	Please refer to the commenting box below
Validity:	Not acceptable

Material and methods:

Test substance:	Triticonazole technical (RPA 400727), batch no. : OP9750057, purity : 90.52%
Test species:	*Selenastrum capricornutum* (*Pseudokirchneriella subcapitata*) stock culture at

	Springborn, originally from University of Texas
Number of organisms:	0.3 x 10^4 cells/mL; 3 replicates each, per treatment and control groups
Type of test, duration:	Static test, 5 days
Applied concentrations:	
Range-finding test	
Nominal:	0.00099, 0.0099, 0.099, 0.99, 9.9 mg ai/L
Mean measured:	0 (control and solvent control), 2.5 mg ai/L
Toxic reference	None
Solvent:	Dimethylformamid
Test conditions:	
Water quality:	Algal Assay Procedure medium prepared with sterile, deionized water
Temperature:	24 - 25 °C
pH:	7.5 (0 h), 8.9 (5 d)
Incubation:	Continuous illumination, 3200 to 5400 lux
Test parameters:	Daily cell count via haemacytometer and a compound microscope and observation of the health of the algal cells. Temperature was measured daily and additionally continuous in a flask of water adjacent to the test flasks. Light intensity was measured at 0 hour and at each 24-hour interval during the exposure period. pH and conductivity were measured at test initiation and at test termination. For chemical analysis of the test substance, samples of test solution, solvent control and control were taken at test initiation and at test termination.
Statistics:	A t-Test (Sokal and Rohlf, 1981) was conducted to statistically compare the treatment level cell density with control and solvent control. EC_{25} and EC_{50} were not calculated, but empirically estimated.
Findings:	
Range- finding test	At test termination, cell densities in the 0.00099, 0.0099, 0.099, 0.99, 9.9 mg ai/L treatment levels averaged 223, 216, 212, 206 and 26 x 10^4 cells/mL.
Analytical data:	The measured test concentrations were 83% of nominal concentrations.

Table 9.2-35: Effects of technical triticonazole on the green algae *Selenastrum capricornutum*

Triticonazole [mg/L] (mean measured)	**Cell density [x 10^4 cells/mL)] (SD)**					
	Observation interval					**% inhibition relative to the pooled control**
	Day 1	**Day2**	**Day 3**	**Day 4**	**Day 5**	
Control	5.8 (1.1)	16 (1.7)	40 (4.4)	101 (1.8)	221 (6.1)	NA
Solvent control	5.2 (1.8)	16 (1.2)	40 (2.4)	102 (1.3)	226 (4.8)	NA
Pooled control	NA	NA	NA	NA	224 (5.6)	NA
2.5	3.7 (0.8)	9.9 (2.2)	25 (0.8)	94 (2.8)	150 (6.2)*	33

SD standard deviation
NA not applicable
*significantly reduced compared to pooled control, based on t-Test

Conclusion:	96 h EC_{50} > 2.5 mg ai/L NOEC < 2.5 mg ai/L based on mean measured concentration.
Comment RMS:	The study was evaluated following the recommendations of the currently valid test guideline OECD 201 (2006) **Check of validity criteria:** - The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period. In the test the cell concentration of the control and solvent control culture increased by a factor of 133 after a 72 h period. Fulfilled. - The mean coefficient of variation for section-by-section specific growth rates (day 0-1, 1-2 and 2-3, for 72-hour tests) in the control cultures must not exceed 35%. No coefficient of variation is derived in the study. - The coefficient of variation of average specific growth rates during the whole test period in replicate control cultures must not exceed 7% in tests with *Pseudokirchneriella subcapitata* (former *Selenastrum capricornutum*). No coefficient of variation was derived in the study. **In addition, the following points deviated from the test guideline or were not reported in detail:** - According to OECD 201 reference substances should be tested at least twice a year. No information is given in the study report about reference testing. - According to OECD 201 a limit test can be conducted at 100 mg/L or a concentration equal to the limit of solubility. The limit of solubility was not determined and the test was conducted only with nominal 3 mg/L. Furthermore the Guideline requires at least six treatment replicates. In this study only three replicates were tested - According to OECD 201 the initial cell density for *Pseudokirchneriella subcapitata* (former *Selenastrum capricornutum*) should be 5 x 10^3 – 10^4 cells/mL. The initial cell density in the test was 0.3 x 10^4 cells/mL. **Acceptability of the analytical methods used in the test:** The evaluation of the analytical methods was conducted according to the Guidance Document SANCO/3029/99 rev. 4. Based on the evaluation (see Volume 3 B.5-CA, B.5.1.2.6) the analytical method is considered valid to quantify the amount of triticonazole in fresh and seawater. LOQ: 0.2493 mg/L **Endpoints:** No E_rC_{50} or E_bC_{50} was reported.

	The Cell density inhibition at termination of the test was 33% based on the mean measured concentration of 2.5 mg/L. **Conclusion of the RMS:** Based on the evaluation of the study the long-term *Selenastrum capricornutum* toxicity test is not considered valid as insufficient information is given in the study report furthermore the limit test was conducted with a lower concentration than recommended in the guidelines and with only three treatment replicates instead of at least six.

Reference:	***Toxicity of Reg. No. 4378513 (Triticonazole technical (BAS 595) to Pseudokirchneriella subcapitata in an Algal Growth Inhibition Test***
Author(s), year:	Seeland-Fremer, A., Wydra V., 2014a
Report/Doc. number:	BASF DocID 2014/1083347
Guideline(s):	OECD 201 (2006), corrected July 28, 2011
GLP:	Yes
Deviations:	Please refer to the commenting box below
Validity:	Acceptable

Material and methods:

Test substance:	Triticonazole technical (BAS 595F), Reg. No. 4378513, batch no. : COD-001440 , purity: 91.3% (analysed)
Test species:	Green algae, *Pseudokirchneriella subcapitata,* cultivated in the laboratories of IBACON; original source: Sammlung von Algenkulturen, Universität Göttingen
Number of organisms:	5000 cells/mL; 3 replicates per treatment group and 6 replicates per control groups. Additionally, one replicate of each test concentration and of the controls was prepared without algae to provide a "blank"
Type of test, duration:	Static test, 72 hours
Applied concentrations:	
Nominal:	0 (control and solvent control), 10, 3.2, 1.0, 0.32 and 0.1 mg ai/L
Solvent:	Dimethyl formamide (DMF), 100 µL/1000 mL
Reference item:	Potassium dichromate – tested at least twice a year
Test conditions:	
Water quality:	Nutrient medium according to the OECD guideline
Temperature:	21.3 – 23 °C
pH:	7.9 -8.0 (start of the test) and 9.3 – 9.8 (end of the test)
Incubation:	Continuous illumination, 5181 – 5960 lux (mean: 5486 lux)
Test parameters:	The cell densities in the samples were determined by spectrophotometrical measurement after 24, 48 and 72 hours of exposure. The algal cell densities were calculated by subtracting the absorption of the blanks,

from each of the measured absorption of the test media (with algae).
The cell densities in a number of samples from one control replicate were counted by microscope after 72 hours of test duration.
The pH was measured in all test item concentrations and the controls at the start and the end of the test.
The temperature was measured daily in an Erlenmeyer flask filed with water and incubated under the same conditions as the test flasks. The light intensity was measured once during the test at 6 positions distributed over the experimental area at the surface of the test media.
The concentrations of the test item were analysed in two of the triplicate test media samples from all test concentrations, control and solvent control samples at 0 and 72 hours.

Statistics: The software used to perform the statistical analysis was ToxRat Professional, Version 2.10.05, ToxRat® Solutions GmbH.

Findings:

Analytical data: The analytical findings of triticonazole in the treatment levels found on day 0 were 96 % to 120 % of nominal (average 106 %). On day 3 analytical findings of 101 % to 109 % of nominal (average 102 %) were found. All results are based on nominal test concentrations.

Table 9.2-36: Effects of triticonazole technical on the green algae *Pseudokirchneriella subcapitata*

triticonazole [mg/L] (nominal)	**Mean cell number per mL (72 h) [1000 cells/ml]**	**Average specific growth rates per day (0-72h)**	**% inhibition of growth rate compared to control**
Control	129.420	1.852	- 0.3
Solvent control	126.999	1.845	-
0.1	125.184	1.841	0.2
0.32	130.429	1.853	-0.4
1.0	140.582	1.879	-1.8
3.2	118.998	1.824	1.2
10	92.841	1.741	5.6*

* Mean value significantly different from the solvent control (tested with Williams t-Test, $\alpha = 0.05$, one-sided)

Conclusion:
72 h E_rC_{10} > 10 mg/L
72 E_yC_{10} = 4.39 mg/L (95% C.I. = 2.86 – 5.46 mg/L)
72 h E_rC_{20} > 10 mg/L
72 E_yC_{20} = 7.54 mg/L (95% C.I. = 6.27 – 8.68 mg/L)
72 h E_rC_{50} > 10 mg/L
72 E_yC_{50} > 10 mg/L
72 h NOEC = 3.2 mg/L (yield and growth rate)

based on nominal concentrations

Comment RMS:	The study was evaluated following the recommendations of the currently valid test guideline OECD 201 (2006) **Check of validity criteria:** - The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period. In the test the cell concentration of the control cultures increased by a factor of 254 after a 72 h period. Fulfilled. - The mean coefficient of variation for section-by-section specific growth rates (day 0-1, 1-2 and 2-3, for 72-hour tests) in the control cultures must not exceed 35%. The coefficient of variation derived in the current study is 24.8%. Fulfilled - The coefficient of variation of average specific growth rates during the whole test period in replicate control cultures must not exceed 7% in tests with *Pseudokirchneriella subcapitata*. The coefficient of variation derived in the current study is 0.9%. Fulfilled. **Acceptability of the analytical methods used in the test:** The evaluation of the analytical methods was conducted according to the Guidance Document SANCO/3029/99 rev. 4. Based on the evaluation (see Volume 3 B.5-CA, B.5.1.2.6) the analytical method was sufficiently validated for determination of triticonazole in freshwater. LOQ: 0.1 mg/L. **Endpoints:** The cell densities were estimated by spectrophotometer. No detailed information was provided about the conditions of this method and about the calibration. The notifier provided some data afterwards which was considered acceptable. The endpoints were recalculated by the RMS via ToxRatPRo® 3.2 as follows: 72 h E_rC_{10} > 10 mg/L 72 E_yC_{10} = 4.36 mg/L (95% C.I. = 2.56 – 7.52 mg/L) reliability based on normalised width of C.I. = poor 72 h E_rC_{20} > 10 mg/L 72 E_yC_{20} = 7.27 mg/L (95% C.I. = 4.00 – 13.54 mg/L) 72 h E_rC_{50} > 10 mg/L 72 E_yC_{50} > 10 mg/L 72 h NOEC = 1.0 mg/L (yield and growth rate) 72 h LOEC = 3.2 mg/L (yield and growth rate) based on nominal concentrations **Conclusion of the RMS:** Based on the evaluation of the study the long-term *Pseudokirchneriella subcapitata* toxicity test is considered valid.

Reference:	**Triticonazole – toxicity to the freshwater diatom, *Navicula pelliculosa***
Author(s), year:	Hoberg, J.R, 1998d
Report/Doc. number:	R012969
Guideline(s):	FIFRA Guideline 122-2 and 123-2
GLP:	Yes
Deviations:	Please refer to the commenting box below
Validity:	Not acceptable

Material and methods:

Test substance:	Triticonazole technical (RPA 400727), batch no.: OP9750057, purity: 90.52%
Test species:	Freshwater diatom, *Nevicula pelliculosa;* Springborn (Wareham) culture
Number of organisms:	1 x 10^4 cells/mL; 3 replicates each, per treatment group and control groups
Type of test, duration:	Static test, 5 days
Applied concentrations:	
Range-finding test	
Nominal:	0.00099, 0.0099, 0.099, 0.99, 9.9 mg ai/L
Definitive test	
Nominal:	0 (control and solvent control), 0.93, 0.39, 0.75, 1.5, and 3 mg ai/L
Mean measured:	0 (control and solvent control), 0.092, 0.17, 0.27, 0.59, 1.2 and 2.5 mg ai/L
Solvent:	Dimethylformamide
Test conditions:	
Water quality:	Algal Assay Procedure medium prepared with sterile, deionized water
Temperature:	25 °C
pH:	7.3-7.5 (0 h), 7.4 – 8.1 (120 h)
Incubation:	Continuous illumination, 4100 - 5400 lux
Test parameters:	Daily cell count via haemacytometer and a compound microscope and observation of the health of the algal cells. Temperature was measured daily and additionally continuously in a flask of water adjacent to the test flasks. Light intensity was measured at 0 hour and at each 24-hour interval during the exposure period. pH and conductivity were measured at test initiation and at test termination. For chemical analysis of the test substance, samples of test solution, solvent control and control were taken at test initiation and at test termination.
Statistics:	A t-Test (Sokal and Rohlf, 1981) was conducted to statistically compare the cell density of the control to the cell density of the solvent control. Dependent on if a statistical difference was determined the solvent control data was used or the control and solvent control data were pooled for further statistical analysis to determine treatment-related effects. The NOEC was determined using Williams' Test (Williams, 1971, 1972).

The 1-, 2-, 3-, 4-, and 5-day EC_{25} and EC_{50} values and the 95% confidence limits were calculated for cell densities by linear regression of response versus exposure concentration over the range of test concentration where a clear exposure-response relationship was observed. A computer program developed and validated at Springborn was used to assist in these computations.

Findings:

Range- finding test: At test termination, cell densities in the 0.00099, 0.0099, 0.099, 0.99, 9.9 mg ai/L treatment levels averaged 97, 95, 94, 72 and 0.13 x 10^4 cells/mL.

Analytical data: The measured test concentrations were in a range of 70 to 99% of nominal concentrations.

Table 9.2-37: Effects of technical triticonazole on the diatom alga *Navicula pelliculosa*

Triticonazole [mg/L] (mean measured)	**Cell density [x 10^4 cells/mL)] (SD)**					
	Observation interval					**% inhibition relative to the pooled control**
	Day 1	**Day2**	**Day 3**	**Day 4**	**Day 5**	
Control	3.1 (0.80)	18 (3.9)	36 (3.4)	102 (2.6)	155 (1.4)	NA
Solvent control	3.4 (0.38)	19 (0.88)	36 (1.8)	101 (2.4)	151 (2.5)	NA
Pooled control	3.3 (0.59)	18 (2.6)	36 (2.4)	102 (2.3)	153 (2.5)	NA
0.092	3.3 (0.9)	15 (3.8)	39 (1.4)	102 (1.4)	150 (1.1)*	1.9
0.17	2.3 (0.50)	15 (0.52)	37 (1.3)	100 (0.76)	149 (3.0)*	2.3
0.27	1.6 (0.38)	11 (2.2)	35 (0.66)	96 (2.5)	143 (2.3)*	6.7
0.59	1.8 (0.38)	13 (1.8)	33 (0.90)	90 (2.1)	122 (2.2)*	21
1.2	0.92 (0.38)	4.0 (0.25)	15 (0.75)	29 (3.3)	46 (2.6)*	70
2.5	0.75 (0.66)	4.8 (0.38)	12 (2.6)	21 (2.3)	33 (1.8)*	78

SD standard deviation
NA not applicable
*significantly reduced compared to pooled control, based on Williams' Test

Conclusion:

24h EC_{25} = 0.17 mg ai/L (95% C.I. 0.017 – 1.1 mg ai/L)
24h EC_{50} = 0.54 mg ai/L (95% C.I. 0.073 – 4.3 mg ai/L)
48h EC_{25} = 0.22 mg ai/L (95% C.I. 0.04 – 0.88 mg ai/L)
48h EC_{50} = 0.67 mg ai/L (95% C.I. 0.15 – 3.0 mg ai/L)
72h EC_{25} = 0.84 mg ai/L (95% C.I. 0.41 – 1.8 mg ai/L)
72h EC_{50} = 1.5 mg ai/L (95% C.I. 0.72– 3.3 mg ai/L)
96h EC_{25} = 0.65 mg ai/L (95% C.I. 0.30 – 1.4 mg ai/L)
96h EC_{50} = 1.1 mg ai/L (95% C.I. 0.52– 2.5 mg ai/L)
120h EC_{05} = 0.23 mg ai/L (95% C.I. 0.11 – 0.47 mg ai/L)
120h EC_{25} = 0.43 mg ai/L (95% C.I. 0.21– 0.88 mg ai/L)
120h EC_{50} = 0.95 mg ai/L (95% C.I. 0.47– 2.0 mg ai/L)
NOEC < 0.092 mg/L
based on mean measured concentrations

Comment RMS:	The study was evaluated following the recommendations of the currently valid test guideline OECD 201 (2006) **Check of validity criteria:** - The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period. In the test the cell concentration of the control and solvent control culture increased by a factor of 35.7 after a 72 h period. Fulfilled. - The mean coefficient of variation for section-by-section specific growth rates (day 0-1, 1-2 and 2-3, for 72-hour tests) in the control cultures must not exceed 35%. No coefficient of variation is derived in the study. The RMS estimated a coefficient of variation of 46.1 via ToxRat® Version 3.1.0. - The coefficient of variation of average specific growth rates during the whole test period (72 hours) in replicate control cultures must not exceed 10% in tests with *Navicula pelliculosa.* No coefficient of variation was derived in the study. The RMS estimated a coefficient of variation of 2.0 after 72 hours by ToxRat® Version 3.1.0. Fulfilled. **In addition, the following points deviated from the test guideline or were not reported in detail:** - According to OECD 201 reference substances should be tested at least twice a year. No information is given in the study report about reference testing. **Acceptability of the analytical methods used in the test:** The evaluation of the analytical methods was conducted according to the Guidance Document SANCO/3029/99 rev. 4. Based on the evaluation (see Volume 3 B.5-CA, B.5.1.2.6) the analytical method is considered valid to quantify the amount of triticonazole in fresh and seawater. LOQ: 0.0225 mg/L **Endpoints:** No E_rC_{50}, E_yC_{50} or E_bC_{50} was reported. The RMS did a statistical re-calculation via ToxRat® Version 3.1.0 and estimated following Endpoints: 72h E_rC_{50} = 4.546 mg ai/L (95% C.I. 2.29 – 8.74 mg ai/L) 120h E_rC_{50} = 3.925 mg ai/L (95% C.I. 2.14 – 6.96 mg ai/L) 72h E_yC_{50} = 1.247 mg ai/L (95% C.I. 0.72 – 2.13 mg ai/L) 120h E_yC_{50} = 0.968 mg ai/L (95% C.I. 0.96 – 0.98 mg ai/L) based on mean measured values **Conclusion of the RMS:** Based on the evaluation of the study the long-term *Navicula pelliculosa* toxicity test is not considered valid as the mean coefficient of variation for section-by-section specific growth rates in the control culture is 46.1% which exceeds the validity criteria of 35%.

Reference:	**Triticonazole – toxicity to the marine diatom, *Skeletonema costatum***
Author(s), year:	Hoberg, J.R., 1998e
Report/Doc. number:	B004429
Guideline(s):	FIFRA Guideline 122-2 and 123-2
GLP:	Yes
Deviations:	Please refer to the commenting box below
Validity:	Acceptable

Material and methods:

Test substance:	Triticonazole technical, batch no. OP9750057, purity : 90.52%
Test species:	*Skeletonema costatum,* stock culture at Springborn, originally obtained from Bigelow Laboratories
Number of organisms:	1 x 10^4 cells/mL; 3 replicates per treatment group and control groups
Type of test, duration:	Static test, 120 hours
Applied concentrations:	
Range-finding test	
Nominal:	0 (control and solvent control), 0.00099, 0.0099, 0.099, 0.99, 9.9 mg ai/L
Definitive test	
Mean measured:	0 (control and solvent control), 0.031, 0.006, 0.10, 0.23, 0.44 and 0.97 mg ai/L
Solvent:	Dimethyl formamide
Test conditions:	
Water quality:	Artificially Enriched Seawater (AES) medium prepared with sterile, filtered, natural seawater, salinity 30 ± 2 g/L
Temperature:	20 - 21 °C
pH:	8.0 – 8.1 (0 h), 8.3 – 8.9 (120 h)
Incubation:	16 hours light, 8 hours darkness, 3200 - 4900 lux
Test parameters:	Daily cell count via hamacytometer and a compound microscope and observation of the health of the algal cells. Temperature was measured continuously in a flask of water adjacent to the test flasks. Light intensity was measured at 0 hour and at each 24-hour interval during the exposure period. pH and conductivity were measured at test initiation and at test termination. For chemical analysis of the test substance, samples of test solution, solvent control and control were taken at test initiation and at test termination.
Statistics:	A t-Test (Sokal and Rohlf, 1981) was conducted to statistically compare the cell density over the control to the cell density of the solvent control. Dependent on if a statistical difference was determined the solvent control data was used or the control and solvent control data were pooled for further statistical analysis to determine treatment-related effects.

The NOEC was determined using Williams' Test (Williams, 1971, 1972).

The 1-, 2-, 3-, 4-, and 5-day EC_{25} and EC_{50} values and the 95% confidence limits were calculated for cell densities by linear regression of response versus exposure concentration of the range of test concentration where a clear exposure-response relationship was observed. A computer program developed and validated at Springborn was used to assist in these computations.

Findings:

Range- finding test: At test termination, cell densities in the 0.00099, 0.0099, 0.099, 0.99, 9.9 mg ai/L treatment levels averaged 154, 152, 149, 3.1 and 0 x 10^4 cells/mL.

Analytical data: The measured test concentrations were in a range of 80 to 110% of nominal concentrations.

Table 9.2-38: Effects of technical triticonazole on the diatom alga *Skeletonema costatum*

Triticonazole [mg/L] (nominal)	**Cell density [x 10^4 cells/mL)] (SD)**					
	Observation interval					**% inhibition relative to the pooled control**
	Day 1	**Day2**	**Day 3**	**Day 4**	**Day 5**	
Control	7.4 (0.76)	18 (1.6)	40 (0.63)	105 (4.0)	155 (2.3)	NA
Solvent control	9.0 (0.50)	20 (1.4)	42 (0.63)	101 (1.3)	156 (3.2)	NA
Pooled control	NA	19 (1.7)	NA	103 (3.2)	156 (2.5)	NA
0.031	8.5 (1.1)	19 (0.88)	42 (1.5)	101 (3.3)	156 (0.84)	-0.07
0.062	7.3 (1.2)	17 (1.5)	40 (0.29)	99 (0.88)	153 (1.0)*	2.0
0.13	6.2 (1.0)	16 (0.5)	38 (1.4)	96 (1.0)	147 (2.5)*	5.9
0.25	4.0 (1.2)	14 (1.0)	36 (1.8)	87 (1.4)	145 (1.6)*	7.0
0.5	4.4 (1.8)	6.3 (1.0)	7.0 (0.66)	13 (1.8)	21 (2.0)*	87
0.99	0.58 (0.80)	1.0 (0.66)	1.4 (0.52)	2.8 (0.25)	3.8 (0.88)*	98

SD standard deviation
NA not applicable
*significantly reduced compared to pooled control, based on Williams' Test

The sensitivity of the test system was verified by testing the reference item potassium dichromate. The EC_{50} values for the growth rate and yield were determined to be 1.387 mg/L (growth rate) and 0.433 mg/L (yield).

Conclusion:

72h EC_{25} = 0.19 mg ai/L (95% C.I. 0.085 – 0.39 mg ai/L)
72h EC_{50} = 0.29 mg ai/L (95% C.I. 0.14– 0.61 mg ai/L)
96h EC_{25} = 0.17 mg ai/L (95% C.I. 0.081 – 0.34 mg ai/L)
96h EC_{50} = 0.26 mg ai/L (95% C.I. 0.13– 0.53 mg ai/L)
120h EC_{25} = 0.21 mg ai/L (95% C.I. = 0.084 – 0.50 mg ai/L)
120 h EC_{50} = 0.31 mg ai/L (95% C.I. = 0.13 – 0.76 mg ai/L)
NOEC = 0.031 mg/L
based on nominal concentrations

Comment RMS:	The study was evaluated following the recommendations of the currently valid test guidelines OCSPP 850.4500 (2012) and OECD 201 (2006) **Check of validity criteria:** OCSPP 850.4500: - All test vessels are identical. Fullfilled. - Treatments are randomly assigned to individual test vessel locations, individual test organisms were randomly assigned to test vessels or compartments. Fulfilled. - A medium (untreated) control (and solvent (vehicle) control, when a solvent was used) was included in the test. A dilution water control and a solvent control with dimethylformamide were tested in the study. Fulfilled. - The concentration of solvent in the range used did not affect growth of the test species. Fulfilled. - During the 96 hour test period, cell counts in the controls did increase by a factor of at least 30 times for *S. costatum*. (*i.e.*, logarithmic growth in the controls was reached during the test). In the test the cell concentration of the control and solvent control culture increased by a factor of 103 after a 96 h period. Fulfilled. - A minimum of five test concentrations were used in the definitive test. In the current test six concentration were tested. Fulfilled. - Controls were not contaminated with the test substance. Fulfilled. - The lowest test concentration level was less than the 96-hour yield, average specific growth rate and area under the growth curve IC_{50} values based on cell density. In the current study the 96-hour yield was 100 and the average specific growth rate was 1.154 and the E_rC_{50} was 0.529 mg/L the lowest tested concentration was 0.031 mg/L. Fulfilled. - For testing with industrial chemicals a surfactant or dispersant was not used in the preparation of a stock or test solution. Fulfilled - Temperature and light intensity were measured as specified during the test. In the current test temperature was measured continuously in a flask of water adjacent to the test flasks. Light intensity was measured at 0 hour and at each 24-hour interval during the exposure period. Fulfilled. OECD 201 (2006): The used test species, the diatom *Skeletonema costatum* is not stated in the test guideline OECD 201 (2006) as proposed test species. Hence, the validity criteria given in the test guidelines have to be considered with caution. - The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period. In the test the cell concentration of the control and solvent control culture increased by a factor of 40 after a 72 h period. Fulfilled. - The mean coefficient of variation for section-by-section specific growth rates

(day 0-1, 1-2 and 2-3, for 72-hour tests) in the control cultures must not exceed 35%. No coefficient of variation is derived in the study. The RMS estimated a coefficient of variation of 60.4 via ToxRat® Version 3.1.0.

- The coefficient of variation of average specific growth rates during the whole test period (72 hours) in replicate control cultures must not exceed 7% in tests with *Pseudokirchneriella subcapitata* and *Desmodesmus subspicatus*. For other less frequently tested species, the value should not exceed 10%. No coefficient of variation is derived in the study. The RMS estimated a coefficient of variation at 72 hours of 1.0 by ToxRat® Version 3.1.0. Fulfilled.

In addition, the following points deviated from the test guideline or were not reported in detail:

- According to OCSPP 850.4500 the test duration is 96 hours. In the current study the test duration was 120 hours.
- According to OCSPP 850.4500 at test termination the coefficient of variation for mean control yield should be less than 15% and it should be less than 15% for average specific growth rate, which is a logarithmically-transformed variable. No coefficients of variation were derived in the study. The RMS estimated a coefficient of variation for the mean control yield of 1.3% and for the average specific growth rate of 0.7 after 96 hours by ToxRat® Version 3.1.0.
- According to OCSPP 850.4500 the minimum number of replicates per treatment and control is four. The number of replicates in the current test was only three.
- According to OECD 201 reference substances should be tested at least twice a year. No information is given in the study report about reference testing.
- According to OECD 201 the number of control replicates ideally should be twice the number of replicates used for each test concentration. In the current test the number of treatment replicates of control replicates is both three.

Acceptability of the analytical methods used in the test: The evaluation of the analytical methods was conducted according to the Guidance Document SANCO/3029/99 rev. 4. Based on the evaluation (see Volume 3 B.5-CA, B.5.1.2.6) the analytical method is considered valid to quantify the amount of triticonazole in fresh and seawater.

LOQ: 0.00874 mg ai/L

Endpoints:

No E_rC_x, E_yC_x or E_bC_x was reported. The RMS did a statistical re-calculation via ToxRat® Version 3.1.0 and estimated following Endpoints:

72h E_rC_{10} = 0.25 mg ai/L (95% C.I. 0.23 – 0.29 mg ai/L)

reliability based on normalised width of C.I. = good

72h E_rC_{20} = 0.31 mg ai/L (95% C.I. 0.28 – 0.35 mg ai/L)

72h E_rC_{50} = 0.46 mg ai/L (95% C.I. 0.40 – 0.53 mg ai/L)

96h E_rC_{10} = 0.23 mg ai/L (95% C.I. 0.20 – 0.26 mg ai/L)
reliability based on normalised width of C.I. = good
96h E_rC_{20} = 0.30 mg ai/L (95% C.I. 0.26 – 0.35 mg ai/L)
96h E_rC_{50} = 0.53 mg ai/L (95% C.I. 0.45 – 0.63 mg ai/L)
120h E_rC_{10} = 0.24 mg ai/L (95% C.I. 0.20 – 0.28 mg ai/L)
reliability based on normalised width of C.I. = good
120h E_rC_{20} = 0.33 mg ai/L (95% C.I. 0.28 – 0.38 mg ai/L)
120h E_rC_{50} = 0.58 mg ai/L (95% C.I. 0.48 – 0.71 mg ai/L)
72h E_yC_{10} = 0.23 mg ai/L (95% C.I. 0.21 – 0.24 mg ai/L)
reliability based on normalised width of C.I. = excellent
72h E_yC_{20} = 0.26 mg ai/L (95% C.I. 0.24 – 0.28 mg ai/L)
72h E_yC_{50} = 0.33 mg ai/L (95% C.I. 0.30 – 0.36 mg ai/L)
96h E_yC_{10} = 0.22 mg ai/L (95% C.I. 0.21 – 0.23 mg ai/L)
reliability based on normalised width of C.I. = excellent
96h E_yC_{20} = 0.25 mg ai/L (95% C.I. 0.23 – 0.26 mg ai/L)
96h E_yC_{50} = 0.31 mg ai/L (95% C.I. 0.30 – 0.34 mg ai/L)
120h E_yC_{10} = 0.25 mg ai/L (95% C.I. 0.23 – 0.26 mg ai/L)
reliability based on normalised width of C.I. = excellent
120h E_yC_{20} = 0.28 mg ai/L (95% C.I. 0.26– 0.29 mg ai/L)
120h E_yC_{50} = 0.34 mg ai/L (95% C.I. 0.32 – 0.36 mg ai/L)

120h NOEC = 0.031 mg ai/L
based on nominal values

Conclusion of the RMS: Based on the evaluation of the study the long-term *Skeletonema costatum* toxicity test is not considered valid according to OECD 201 as the mean coefficient of variation for section-by-section specific growth rates in the control culture is 60% which exceeds the validity criteria of 35%. However, the used test species, the diatom *Skeletonema costatum* is not stated in this test guideline. According to OCSPP 850.4500 it seems to be valid as for this guideline the section-by-section specific growth rate is not a validity criterion. The study is considered valid to be used in the risk assessment.

Metabolites:

Reference:	**Effects of Reg. No. 5079359 (RPA 406203, Metabolite of BAS 595F, Triticonazole) on the Growth of the Green Alga *Pseudokirchneriella subcapitata***
Author(s), year:	Hoffmann, F., 2009a
Report/Doc. number:	BASF DocID 2009/1050280
Guideline(s):	OECD 201
GLP:	Yes
Deviations:	Please refer to the commenting box below
Validity:	Acceptable

Material and methods:

Test substance:	Reg.no. 5079359, batch no. : BESS0578, purity : 99.9%
Test species:	Green algae, *Pseudokirchneriella subcapitata,* in-house cultures, originally from Sammlung von Algenkulturen, Göttingen
Number of organisms:	1 x 10^4 cells/mL; 5 replicates per treatment group and 10 replicates for the control group,
Type of test, duration:	Static test, 72 hours
Applied concentrations:	
Nominal:	0 (control), 1.56, 3.13, 6.25, 12.5, 25, 50 and 100 mg/L
Mean measured:	0 (control), 1.40, 2.54, 5.03, 10.93, 18.4, 40.5 and 79.5 mg/L
Solvent:	None
Reference item:	Potassium dichromate
Test conditions:	
Water quality:	Nutrient medium according to the OECD guideline
Temperature:	22 ± 1 °C
pH:	7.68 – 8.01
Illumination:	uniform lightning, approx. 8000 lux (no measurement provided)
Test parameters:	Cells concentration in each flask was determined 24, 48 and 72 hours after the start of the test with a spectrophotometer (623 nm, 5 cm glass cuvettes). For the control and the nominal test concentration algal medium without algae were used as a blank. To obtain the actual number of cells/ML a linear correlation (calibration curve) was calculated from the cell numbers (counted under a microscope) versus extinction values. The pH was measured at the last sampling interval of analytical sampling. No information is given about the measurement of temperature and illumination. At the beginning and at the end of the test samples were taken for verification of the test item concentrations.

Statistics: Statistical analysis was performed using the software ToxRat® Professional 2.10.

Findings:

Analytical data: The measured concentrations of Reg.No. 5079359 in the test solution remained within a range of 68.2 – 102.5% of the nominal values at the start of the test and within a range of 68.3-106.6% of the nominal values at the end of the test. Therefore, the toxicity results are based on the mean measured concentrations.

Table 9.2-39: Effects of metabolite Reg. No. 5079359 on the green algae *Pseudokirchneriella subcapitata*

Reg. No. 5079359 [mg/L] (mean measured)	% inhibition of yield compared to control	% inhibition of growth rate compared to control
Control	-	-
1.40	-1.6	-0.4
2.54	11.1	2.7
5.07	33.4	9.4
10.9	54.5	18.2
18.4	79.1	35.7
40.5	82.2	40.3
79.5	87.6	46.9

The sensitivity of the test system was verified by testing the reference item potassium dichromate. The EC_{50} values for the growth rate and yield were determined to be 1.32 mg/L (growth rate) and 0.40 mg/L (yield).

Conclusion: 72 h E_rC_{50} = 73.32 mg/L (95% C.I. = 61.4 – 91.0 mg/L)
72 E_rC_{10} = 4.29 mg/L (95% C.I. = 3.08 – 5.56 mg/L)

72 h E_yC_{50} = 9.29 mg/L (95% C.I. = 8.44 – 10.22 mg/L)
72 E_yC_{10} = 1.99 mg/L (95% C.I. = 1.59 – 2.39 mg/L)
based on mean measured concentrations

Comment RMS: The study was evaluated following the recommendations of the currently valid test guideline OECD 201 (2006)

Check of validity criteria:

- The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period. In the test the cell concentration of the control culture increased by a factor of 72.1 after a 72 h period. Fulfilled.
- The mean coefficient of variation for section-by-section specific growth rates (day 0-1, 1-2 and 2-3, for 72-hour tests) in the control cultures must not exceed 35%. The coefficient of variation derived in the current study is 14.7%. Fulfilled.
- The coefficient of variation of average specific growth rates during the whole test

period in replicate control cultures must not exceed 7% in tests with *Pseudokirchneriella subcapitata*. The coefficient of variation derived in the current study is 0.3%. Fulfilled.

In addition, the following points deviated from the test guideline or were not reported in detail:

- The study report does not provide detailed information about the measurements of temperature and illumination.

Acceptability of the analytical methods used in the test: The evaluation of the analytical methods was conducted according to the Guidance Document SANCO/3029/99 rev. 4. Based on the evaluation (see Volume 3 B.5-CA, B.5.1.2.6) it could be demonstrated that the method fulfils the requirements with regard to linearity, specificity, accuracy, repeatability, and limit of quantification.

LOQ: 0.001 mg ai/ L

Endpoints: The cell densities were estimated by spectrophotometer. No detailed information was provided about the calibration. No raw data was given. The notifier provided some data afterwards. Based on these data the endpoints were recalculated by the RMS via ToxRat® 3.1.0 as follows:

72 E_rC_{10} = 3.51 mg/L (95% C.I. = 2.40 – 5.14 mg/L)

reliability based on normalised width of C.I. = fair

72 E_rC_{20} = 9.55 mg/L (95% C.I. = 6.47 – 14.11 mg/L)

72 h E_rC_{50} = 64.83 mg/L (95% C.I. = 37.84 – 107.19 mg/L)

72 E_yC_{10} = 1.84 mg/L (95% C.I. = 1.45 – 2.35 mg/L)

reliability based on normalised width of C.I. = good

72 E_yC_{20} = 3.12 mg/L (95% C.I. = 2.46 – 3.96 mg/L)

72 h E_yC_{50} = 8.57 mg/L (95% C.I. = 6.46 – 11.39 mg/L)

72 h NOEC = 1.4 mg/L (yield)

based on mean measured concentrations

Conclusion of the RMS:

Based on the evaluation of the study the long-term *Pseudokirchneriella subcapitata* toxicity test is considered valid.

Regarding the study with the intermediate [redacted] the notifier provided following statement:

[redacted] *is an intermediate of the manufacturing process of triticonazole. It is used as a starting material in the first reaction step. Due to the reaction conditions and the purification steps no residual* [redacted] *can be transferred into the technical material. The algal study with this intermediate was conducted in the context of the REACH regulation. However, since the intermediate* [redacted] *) is not present in the final TGAI and will not be formed under natural conditions (it was not measured in any of the environmental fate studies with triticonazole), it is not relevant for the environmental risk assessment of plant protection products under Regulation EC 1107/2009. Finally, it should be noted that the GLP-study is not suitable for the regulatory risk assessment of plant protection products anyway because the tested*

concentrations were not verified analytically.

For the sake of completeness a short study summary based on the summary provided by the notifier is presented below:

Reference:	**Toxicity of [redacted] to *Scenedesmus subspicatus* CHODAT**
Author(s), year:	Peters, 1992a
Report/Doc. number:	C039712
Guideline(s):	OECD 201 (1984)
GLP:	No
Deviations:	None
Validity:	Not acceptable

Material and methods:

Test substance: [redacted]) (BAS 555 F; Reg. no.: 4 539 595; intermediate), batch no: DA 696; purity: 956 g/kg.

Test species: Unicellular fresh water green alga, *Scenedesmus subspicatus* CHODAT (syn. *Desmodesmus subspicatus*); specification: SAG 86.81; stock obtained from "Sammlung von Algenkulturen", Göttingen, Germany.

Type of test, duration: Static system (96 hours); 6 test concentrations plus a control and a solvent control with 4 replicates for each; daily assessment of growth.

Applied concentrations:

Control, solvent control (0.1 g/L Tween-80), 5.00, 10.00, 20.00, 40.00, 80.00, 160.00 mg DMBCP/L (nominal).

Test conditions:

250 mL Erlenmeyer flasks; nutrient solution according to OECD 201; pH 8.8 - 8.9 at test initiation and pH 9.3 - 10.4 at test termination; water temperature: 22.5 ± 1.0°C; initial cell densities: 10000 cells/mL; continuous light, light intensity: 8000 lux, shaking of test tubes three times daily.

Analytics: No analytical verification of test item concentrations was conducted.

Statistics: Descriptive statistics, probit analysis for determination of ECx values.

Findings:

Analytical data: No analytical verification of test item concentrations was conducted. The following biological results are based on nominal concentrations.

Biological effects: Morphological effects on algae were not assessed. Statistically significant effects compared to the control on growth rate and biomass were not evaluated.

Table 9.2-40: Effect of DMBCP on the growth of the green alga *Scenedesmus subspicatus*

Concentration [mg/L] (nominal)	Control	Solvent control	5.00	10.00	20.00	40.00	80.00	160.00
Inhibition in 72 h (growth rate) [%]	14.92	--	11.65	21.96	38.62	63.03	76.62	67.48
Inhibition in 72 h (biomass) [%]	23.68	--	34.40	53.34	72.01	85.93	91.64	91.09
Inhibition in 96 h (growth rate) [%]	8.80	--	6.16	12.76	33.41	62.99	67.16	51.58
Inhibition in 96 h (biomass) [%]	30.88	--	30.18	49.52	74.70	90.82	94.09	92.91
Endpoints [mg DMBCP/L] (nominal) *								
E_rC_{50} (72 h)	35.41							
E_rC_{10} (72 h)	3.44							
E_bC_{50} (72 h)	8.08							
E_bC_{10} (72 h)	0.76							
E_rC_{50} (96 h)	56.38							
E_rC_{10} (96 h)	5.54							
E_bC_{50} (96 h)	8.64							
E_bC_{10} (96 h)	1.14							

*Based on arithmetical evaluation (probit analysis).

Conclusion: In a 96-hour algae test with Scenedesmus subspicatus the ErC_{50} for DMBCP was determined to be 56.38 mg/L, the EbC_{50} was 8.64 mg/L based on nominal concentrations. After 72 hours the ErC_{50} value for DMBCP was determined to be 35.41 mg/L and the EbC_{50} was 8.08 mg/L (nominal).

Comment RMS: The study was not evaluated by the RMS as it is not considered of relevance for the evaluation of the active substance triticonazole. Please also refer to the argumentation provided by the applicant above.

B.9.2.8. Effects on aquatic macrophytes

For the first EU approval of the active substance triticonazole a laboratory study with the aquatic macrophyte *Lemna gibba* was submitted. This study has been re-evaluated by the RMS and a study summary is given below.

Reference:	**Triticonazole – toxicity to the duckweed, *Lemna gibba***
Author(s), year:	Hoberg, J.R., 1998b
Report/Doc. number:	R012023
Guideline(s):	FIFRA Guideline 122-2 and 123-2
GLP:	Yes
Deviations:	Please refer to the commenting box below
Validity:	Not acceptable

Material and methods:	
Test substance:	Triticonazole technical, batch no.: OP9750057, purity: 90.52%
Test species:	*Lemna gibba*, Springborn culture
Number of organisms:	3 replicates per controls and treatments, 5 plants with 3 fronds each per replicate
Type of test, duration:	Semi-static with renewal of the test media on days 0, 3, 6, 9 and 12; 14 days
Applied concentrations:	
Range-finding test	
Nominal	0 (control and solvent control) 0.00099, 0.0099, 0.099, 0.99 and 9.9 mg ai/L
Tier 1 test	
Nominal	0 (control and solvent control) and 3.0 mg ai/L
Tier 2 - definitive test	
Nominal:	0 (control and solvent control), 0.19, 0.39, 0.75, 1.5, and 3.0 mg ai/L
Measured (mean):	0 (control and solvent control), 0.18, 0.33, 0.65, 1.3, and 2.8 mg ai/L
Solvent:	Dimethyl formamide
Test conditions:	
Water quality:	Hoagland's medium
Temperature:	25 °C
pH:	5.0 – 6.1
O_2 content:	Not given
Light regime:	Continuous light, 4200 – 4600 lux
Test parameters:	At each 3-day interval and at test termination (day 14), fronds were counted and observations were made. At test termination, frond densities for each replicate treatment, control and solvent control vessel were determined. Fronds were counted and removed from each vessel and dried at 76 °C for three days for dry weight determination. pH-values were measured at test initiation, at each 3-day interval and at test

termination. The temperature was measured and recorded continuously in a flask of water adjacent to the test vessels within the environmental chamber.

Light intensity was determined at test start and at each subsequent daily interval during the exposure period.

Analytical measurements: At the beginning and end of one renewal period, one sample was removed from each treatment, control and solvent control solution for analysis of test concentration.

Statistics: Means and standard deviations of frond densities and for biomass (based on dry plant weight at test termination). A t-Test (Sokal and Rohlf, 1981) was used to compare the 14-day control and solvent control growth rate and biomass data. Percent inhibition of the 14-day mean frond density and biomass of treatment data were calculated relative to the pooled control data.

EC_{25} and EC_{50}-value were determined by linear regression of response versus mean measured test concentration. A computer program was used to assist in these computations.

NOEC-values were determined by using Williams' Test (Williams, 1971, 1972).

Findings:

Analytical data: The mean measured concentrations of triticonazole were in a range of 84 – 95% of nominal test concentrations. However, the results are based on mean measured test concentrations.

Table 9.2-41: Mean yield for plant shoots, frond densities and dry weights

Triticonazole [mg/L] (mean measured)	**Frond number at day 14 (SD)**	**Mean biomass integrals**	
		Biomass at day 14 [g]	**% inhibition relative to pooled control**
Control	569 (106)	0.1101 (0.0411)	NA
Solvent control	513 (141)	0.1055 0.0332)(	NA
Pooled control	541 (115)	0.108 (0.0335)	NA
0.18	611 (69)	0.1083 (0.0201)	-0.46
0.33	498 (15)[b]	0.0971 (0.0027)	9.9
0.65	389 (59)[abc]*	0.0699 (0.0192)*	35
1.3	274 (12) [abc]*	0.0440 (0.0057)*	59
2.8	178 (9.1)[abcd]*	0.0267 (0.0019)*	75

SD standard deviation; NA not applicable
[a] Slightly chlorotic fronds were observed
[b] Fronds were smaller in comparison to the control
[c] Less root formation was observed in comparison to the control
[d] Curled fronds were observed
* Statistically significant difference from control, Williams' Test

Conclusion: 14 d $EC_{25\ (frond\ density)}$ = 0.61 mg ai/L (95% C.I. = 0.36 – 1.1 mg ai/L)

14 d $EC_{50\ (frond\ density)}$ = 1.4 mg ai/L (95% C.I. = 0.84 – 2.5 mg ai/L)
14 d $E_bC_{25\ (dry\ weight)}$ = 0.46 mg ai/L (95% C.I. = 0.19 – 1.1 mg ai/L)
14 d $E_bC_{50\ (dry\ weight)}$ = 1.1 mg ai/L (95% C.I. = 0.47 – 2.7 mg ai/L)
Based on mean measured concentrations

Comment RMS:	The study was evaluated following the recommendations of the currently valid test guideline OECD 221 (2006) **Check of validity criteria:** - The doubling time of frond number in the control must be less than 2.5 days (60h), corresponding to approximately a seven-fold increase in seven days and an average specific growth rate of 0.275 d^{-1}. The doubling time in the current test was 2.6 days, corresponding to a 11.3 – fold increase in nine days and an average specific growth rate of 0.269 d^{-1}. Fulfilled. **In addition, the following points deviated from the test guideline or were not reported in detail:** - According to OECD 221 a reference substance should be tested at least twice a year. No information about reference testing is provided in the current study report. - According to OECD 221 each test vessel should contain a total of 9 to 12 fronds at test initiation. In the current test each vessel contained 15 fronds. - According to OECD 221 to estimate the dry weight, it should be determined at the start of the test from a sample of the inoculum culture representative of what is used to begin the test, and at the end of the test with the plant material from each test and control vessel. In the current test dry weight was only measured at the end of the test. Other parameters recommended in the guidelines like, total frond area or fresh weight, have not been determined. - According to OECD 221 determination of test substance concentrations prior to renewal need to be performed on one replicate vessel at each test concentration. The results in the study do not differentiate between measurements in newly prepared solution and measurements in old solution. The limit of quantification was determined to be 0.0225 mg ai/L. The concentrations measured in the control and solvent control respectively are reported to be < 0.024 mg ai/L. **Acceptability of the analytical methods used in the test:** Evaluation of the analytical methods (water/sediment/soil) according to the Guidance Document SANCO/3029/99 rev. 4. Method: HPLC Linearity: Calibration is based on duplicate determination at five concentrations.

	The calibration function was linear within the range from 0.500 – 5.00 mg ai/ with r^2 = 0.999844. Accuracy: 3 fortifications levels (each 3 measurements): 0.0792, 0.396 and 1.98 mg/L; mean recoveries for each level: 97.3-106% Precision: The relative standard deviation per fortification level ≤ 20 % LOQ: 0.0225 mg ai/L LOD: not given The analytical method fulfils the requirements of guideline SANCO/3029/99 rev. 4. **Endpoints:** No E_rC_x and E_yC_x values were provided in the study report based on mean measured concentrations **Conclusion of the RMS:** As according to the study report concentrations of active substance were measured in the control and in the solvent control, the study is considered not valid.

B.9.2.9. Further testing on aquatic organisms

No further testing was provided.

B.9.3. EFFECTS ON ARTHROPODS

B.9.3.1. Effects on bees

B.9.3.1.1. Acute toxicity to adult honeybees

Studies on the acute oral and contact toxicity of triticonazole were already submitted for the first EU approval of the active substance. In addition, new acute oral and contact toxicity studies were submitted addressing the acute risk to adult honey-bees.

Reference:	**Laboratory testing for toxicity (acute contact and oral LD_{50}) of triticonazole on honey bees (*Apis mellifera* L.) (Hymenoptera, Apidae)**
Author(s), year:	Schmitzer, S., 1998
Report/Doc. number:	R005760
Guideline(s):	EPPO 170 (1992)
GLP:	Yes
Deviations:	Uptake of test substance in oral test took 6 hours instead of the indicated 3 hours in one of the replicates in the 100 µg/bee treatment group – According to the study author this was considered not to affect the study as no bee died in the oral test. For further deviations please refer to the commenting box below
Validity:	Acceptable

Material and Methods:

Test substance:	Triticonazole., Batch no.: 9802018, Purity: 965 g/kg (analytical)
Reference:	Danadim 400 g/L Dimethoate EC Formulation
Solvent:	Acetone
Test species:	*Apis mellifera* L., adult worker honeybees, 4-6 week old female bees; honey bee colonies, disease-free and queen-right, bred by IBACON
Type of test:	Acute oral and contact toxicity test
Number of organisms:	Three replicates with 10 bees for control and the test item treatment groups
Food:	Commercial ready-to-use syrup (e.g. Apiinvert, Co. Südzucker AG) *ad libitum*
Oral toxicity test:	
Applied concentrations:	Solvent control Test item nominal: 6.25, 12.5, 25.0, 50.0 and 100 µg ai/bee Test item consumed: 9.5, 20.3, 38.9, 77.2 and 155.5 µg ai/bee
Toxic standard:	0.3 µg//bee
Exposure route:	Ca. 30 mg of triticonazole per bee, mixed in food (1 part solvent + 19 parts syrup) and offered in syringes which are weighed before and after introduction into the cages (duration of uptake was 3 hours, except in one dosage group, see deviations

to the protocol).

Test conditions: Temperature: 28-29 °C, relative humidity: 48 - 66 %, darkness (except during observations, ventilation to avoid possible accumulation of pesticide vapour

Test parameter: Mortality: number of dead bees after 30, 45, 60 minutes; 2, 4 hours (first day); 24 and 48 hours.

Behavioural abnormalities (vomiting, apathy, intensive cleaning): after 30, 45, 60 minutes; 2, 4 hours (first day); 24 and 48 hours.

Contact toxicity test:

Applied concentrations: CO_2 and CO_2/solvent treated control

Test item: 6.25, 12.5, 25, 50 and 100 µg ai/bee

Toxic standard 0.2 µg/bee

Exposure route: 5 µL of the appropriate solution of the test item dissolved in acetone was placed on the ventral bee thorax using a Burkard-Applicator.

Test conditions: Temperature: 28-29 °C, relative humidity: 48 - 66 %, darkness (except during observations, ventilation to avoid possible accumulation of pesticide vapour

Test parameter: Mortality: number of dead bees after 30, 45, 60 minutes; 2, 4 hours (first day); 24 and 48 hours.

Behavioural abnormalities (vomiting, apathy, intensive cleaning): after 30, 45, 60 minutes; 2, 4 hours (first day); 24 and 48 hours.

Findings:

Oral toxicity test: None of the 150 bees died after ingestion of triticonazole by the end of the experiment. During application day in the 155.5, 77.2, 38.9 and 20.3 µg/bee dosage groups the bees were sitting in one corner of the test cage and did not move. After a short push they moved around, but assembled themselves in one corner after a short while.

For the toxic standard one dose (0.3 µg/bee) was tested resulting in 66.7% mortality after 24 hours.

Table 9.3-1: Behavioural abnormalities[a] of the bees in the oral toxicity test

Consumed test substance [µg ai/bee]	Time after ingestion				
	30 minutes	1 hour	4 hours	24 hours	48 hours
	Behavioural abnormalities [mean%]				
155.5	0.0	100.0	80.0	0.0	0.0
77.2	0.0	83.3	100.0	0.0	0.0
38.9	0.0	60.0	83.3	0.0	0.0
20.3	0.0	13.3	0.0	0.0	0.0
9.5	0.0	0.0	0.0	0.0	0.0
Solvent	0.0	0.0	0.0	0.0	0.0
Toxic standard	0.0	0.0	3.3	13.3	6.7

[a]results are averages from three replicates (ten bees each) per dosage/control

Contact toxicity test: In the 100, 50, and 6.3 µg/bee treatment group, each, one bee died by the end of the experiment (3.3%). In the 12.5 µg/bee treatment group two bees died (6.7%). A few bees showed behavioural

abnormalities in all treatment groups except 25 µg/bee treatment group, like discoordinated movement and lethargy.

No bee died in the solvent/CO_2 control as well as in the CO_2-treated negative controls within the entire experimental time.

For the toxic standard one dose (0.2 µg/bee) was tested resulting in 100% mortality after 24 hours.

Table 9.3-2: Mortalities[a] of the bees in the contact toxicity test

Nominal dosage [µg ai/bee]	Time after ingestion				
	30 minutes	1 hour	4 hours	24 hours	48 hours
	Mortality [mean %]				
100	0.0	0.0	0.0	0.0	3.3
50	0.0	0.0	0.0	0.0	3.3
25	0.0	0.0	0.0	0.0	0.0
12.5	0.0	0.0	0.0	6.7	6.7
6.3	0.0	0.0	0.0	0.0	3.3
CO_2 treated control	0.0	0.0	0.0	0.0	0.0
CO_2/solvent control	0.0	0.0	0.0	0.0	0.0
Toxic standard	0.0	0.0	36.7	100.0	100.0

[a]results are averages from three replicates (ten bees each) per dosage/control

Table 9.3-3: Behavioural abnormalities[a] of the bees in the contact toxicity test

Nominal dosage [µg ai/bee]	Time after ingestion				
	30 minutes	1 hour	4 hours	24 hours	48 hours
	Behavioural abnormalities [mean %]				
100	0.0	3.3	3.3	3.3	0.0
50	0.0	3.3	0.0	0.0	0.0
25	0.0	0.0	0.0	0.0	0.0
12.5	100.0	10.0	6.7	0.0	0.0
6.3	0.0	36.7	3.3	3.3	0.0
CO_2 treated control	0.0	0.0	0.0	0.0	0.0
CO_2/solvent control	0.0	0.0	0.0	0.0	0.0
Toxic standard	0.0	0.0	53.3	0.0	0.0

[a]results are averages from three replicates (ten bees each) per dosage/control

Conclusions:

48 h LD_{50} > 155.5 µg ai/bee (oral toxicity)

48 h LD_{50} > 100 µg ai/bee (contact toxicity)

Comment RMS:	The study was evaluated following the recommendations of the currently valid test guideline OECD 213 (1998) for oral toxicity and OECD 214 (1998) for contact

toxicity

Check of validity criteria:

- The average mortality for the total number of controls must not exceed 10% at the end of the test. In the current test no mortalities occurred in the controls. Fulfilled.
- The LD_{50} of the toxic standard meets the specified range (0.10-0.35 µg dimethoate/bee for the oral test and 0.1-0.3 µg dimethoate/bee for contact test). In the current study the LD_{50} for the toxic standard was < 0.2 and < 0.3 µg dimethoate/bee for oral and contact testing, respectively. Fulfilled.

In addition, the following points deviated from the test guideline or were not reported in detail:

- According to OECD 213 and OECD 214 a reference substance should be tested with at least three doses to cover the expected LD_{50} value. In the current study only one dosage with 0.3 µg dimethoate/bee for the oral test, and 0.2 µg dimethoate/bee for the contact test, was chosen for the toxic standard.
- According to OECD 213 and OECD 214 the bees should be held at a temperature of 25±2°C. The relative humidity should be around 50-70% and be recorded throughout the test. In the current study the temperature was 28-29°C and the relative humidity was 48-66°C. No information is given about the method and continuity of measurement. IBACON states that experience of IBACON has shown that this deviation to the guideline will have no adverse effect on the study.
- According to OECD 213 the concentration of the solvent must be given. In the current study Acetone was used as solvent, however no concentration was reported.
- According to OECD 213 the bees may be starved for up to 2 hours before the initiation of the test. In the current study no information is reported about starvation before test initiation.
- According to OECD 214 a volume of 1 µl of solution containing the test substance at the suitable concentration should be applied. In the current study, 5 µl of the test substance in solvent was applied.

Acceptability of the analytical methods used in the test: No information regarding the validation of the analytical methods was reported.

Endpoints:

The RMS agrees on the endpoints given in the study report.

48 h LD_{50} > 155.5 µg ai/bee (oral toxicity)

48 h LD_{50} > 100 µg ai/bee (contact toxicity)

Conclusion of the RMS: Based on the evaluation of the study the acute oral and contact honey bee toxicity test is considered valid.

Reference:	**Effects of BAS 595 F (acute contact and oral) on honey bees (*Apis mellifera* L.) in the laboratory**
Author(s), year:	Hernádi, D., 2006a
Report/Doc. number:	BASF DocID: 2006/1024251
Guideline(s):	OECD 213 and 214 (1998)
GLP:	Yes
Deviations:	Please refer to the commenting box below
Validity:	Acceptable

Material and Methods:

Test substance:	Triticonazole., Batch no.: COD-000601, Purity: 90.3% (according to the certificate of analysis)
Certificate of Analysis Ref. Code/Date:	199618_1; 17.07.2006
Reference:	BI 58 EC (nominal: 400 g/L Dimethoate)
Control:	Oral test: sucrose solution (50 w/v %) with deionized water Contact test: deionized water control with anaesthetization and solvent control with anaesthetization
Solvent:	Acetone solution (100 v/v %)
Test species:	*Apis mellifera* L., adult worker honeybees, approx. 6 week old female bees; honey bee colonies, disease-free and queen-right, bred by Györgyi Purger Pordánné
Type of test:	Acute oral and contact toxicity limit test
Number of organisms:	Five replicates with 10 bees for controls and the test item treatment groups
Food:	50 w/v% sucrose solution
Range finding test:	A preliminary range finding test was performed under non-GLP conditions before the main test. The number of treated bees was 20 per test groups. These results were used to establish the definitive dosage levels
Oral toxicity test:	
Applied concentrations:	100 µg ai/bee Water/sugar control 0.07, 0.12, 0.19 and 0.30 µg reference substance/bee
Exposure route:	The test item was dispersed in 50% w/v sucrose solution in appropriate concentration and was administered as a single dose of 0.2 ml in the feeders, which were weighed before and after introduction into the cages (after maximum 6 hours the test item treated food was replaced by fresh, untreated food).
Test conditions:	Temperature: 23.8-25.9 °C, relative humidity: 55 - 67 %, darkness (except during observations), ventilation to avoid possible accumulation of pesticide vapour. Test conditions were recorded manually.
Test parameter:	Mortality: number of dead bees after 4 hours (first day); 24 and 48 hours.

	Behavioural abnormalities (vomiting, apathy, intensive cleaning): after 4 hours (first day); 24 and 48 hours.
Contact toxicity test:	
Applied concentrations:	100 µg ai/bee Water control/solvent control 0.07, 0.12, 0.19 and 0.30 µg reference substance/bee
Exposure route:	Bees were lightly anaesthetised with CO_2 and 2 µL/bee volume of the test item solution and 1 µl/bee volume of the toxic standards solution was applied to the thorax of each bee.
Test conditions:	Temperature: 23.8-25.9 °C, relative humidity: 55 - 67 %, darkness (except during observations), ventilation to avoid possible accumulation of pesticide vapour. Test conditions were recorded manually.
Test parameter:	Mortality: number of dead bees after 4 hours (first day); 24 and 48 hours. Behavioural abnormalities (vomiting, apathy, intensive cleaning): after 4 hours (first day); 24 and 48 hours.

Findings:

Oral toxicity test: No mortality was observed at the actual intake of 96.26 µg ai/bee. No control mortality occurred. No behavioural impairments were observed in the test concentration. The LD_{50} for the toxic standard was 0.1 µg /bee

Contact toxicity test: No mortality was observed at the application of 100 µg ai/bee and no control mortality occurred. No behavioural impairments were observed in the test concentration. The LD_{50} for the toxic standard was 0.13 µg /bee

Conclusions:

48 h LD_{50} > 96.26 µg ai/bee (oral toxicity)

48 h LD_{50} > 100 µg ai/bee (contact toxicity)

Comment RMS:	The study was evaluated following the recommendations of the currently valid test guideline OECD 213 (1998) for oral toxicity and OECD 214 (1998) for contact toxicity **Check of validity criteria:** - The average mortality for the total number of controls must not exceed 10% at the end of the test. In the current test no mortalities occurred in the controls. Fulfilled. - The LD_{50} of the toxic standard meets the specified range. (0.1.-0.35 µg dimethoate/bee). In the current study the LD_{50} was 0.10 and 0.13 µg dimethoate/bee for oral and contact toxicity standard, respectively. Fulfilled. **In addition, the following points deviated from the test guideline or were not reported in detail:**

	- According to OECD 213 the bees may be starved for up to 2 hours before the initiation of the test. In the current study starvation time is only stated as: "as appropriate". - According to OECD 214 a volume of 1 µl of solution containing the test substance at the suitable concentration should be applied. In the current study, 2 µl of the test substance in solvent was applied. **Acceptability of the analytical methods used in the test:** No Information regarding the validation of the analytical methods was reported. **Endpoints:** The RMS agrees on the endpoints given in the study report. 48 h LD_{50} > 96.26 µg ai/bee (oral toxicity) 48 h LD_{50} > 100 µg ai/bee (contact toxicity) **Conclusion of the RMS:** Based on the evaluation of the study the acute oral and contact honey bee toxicity test is considered valid.

B.9.3.1.2. Chronic toxicity to adult honeybees

According to the data requirements for active substances (Commission Regulation (EU) 283/2013) and/or plant protection products (Commission Regulation (EU) 284/2013) the chronic risk to adult honeybees has to be evaluated. The applicant addressed the chronic toxicity with a formulation study only. Please refer to Volume 3, B.9-CP). This is considered acceptable by the RMS.

B.9.3.1.3. Effects on honeybee development and other honeybee life stages

No chronic honeybee larvae test test with the active substance was submitted. The applicant addressed the chronic toxicity to honeybee larvae with a formulation study only. Please refer to the Volume 3, B.9-CP.

B.9.3.1.4. Cage and tunnel tests

Based on the results reported in the available laboratory studies (acute oral and contact toxicity to honey-bees, chronic toxicity to adult honeybees and effects on larvae of honey-bees), no further studies are required addressing the risk to honeybees.

B.9.3.1.5. Field tests

Based on the results reported in the available laboratory studies (acute oral and contact toxicity to honey-bees, chronic toxicity to adult honeybees and effects on larvae of honeybees), no further studies are required addressing the risk to honeybees.

B.9.3.1.6. Investigation of special effects

Based on the results on honeybees (adults and larvae) and non-target arthropods it was demonstrated that the active substance shows no insecticidal activity. Hence, no further data are required.

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

No studies with the active substance triticonazole were submitted addressing the risk to non-target arthropods other than bees. Please refer to the Volume 3, B.9-CP.

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

B.9.4.1. Earthworm – acute effects

For the first EU approval of the active substance triticonazole acute earthworm studies with the active substance and its metabolites were submitted addressing the risk to earthworms. According to the current data requirements for active substances (Regulation No 283/2013) and plant protection products (Regulation No 284/2013) acute toxicity studies are no longer required. Nevertheless, the study summaries from the DAR are included in the RAR as additional information.

Handley J.W., Wetton P.M., 1991 – The acute toxicity of RPA 400727 to earthworms (Eisenia foetida)

Document number: R013015

Guideline: OECD Guidelines for Testing of Chemicals (1984), No. 207

GLP: Yes

Material and methods:

A limit test was conducted at the highest recommended test concentration of 1000 mg/kg of soil. 6 replicates of 10 worms each were tested. 4 replicates each of control and each of 5 positive control groups (Chloracetamid) ranging from 5.6 to 56 mg/kg soil were also run to ensure the sensitivity of the test system.

The following artificial soil was used: *Industrial quartz sand* *69* *(% w/w)*

Kaolinite clay *20*

Sphagnum moss peat *10*

Calcium carbonate *1* → *pH 5.8 ±0.1*

The water content of basic substrate at day 0 was adjusted to 30-31% dw. At study end (14 d) the water content amounted for 24-26%. The test was conducted at 21°C.

Findings:

Cumulative mortality data are give in the table below:

Table B.9.6.1-1: Cumulative mortality data for Eisenia foetida (test group 60 worms, all others 40 worms)

	Cumulative mortality		*% Mortality*		*mean worm weight (g)*	
	Day 7	*Day 14*	*Day 7*	*Day 14*	*Day 0*	*Day 14*
control	*0*	*0*	*0*	*0*	*0.47*	*0.46*
Triticonazole 1000 mg/kg	*0*	*1*	*0*	*2*	*0.45*	*0.45*
control	*0*	*0*	*0*	*0*		
chloracetamide 5.6 mg/kg	*0*	*0*	*0*	*0*		
chloracetamide 10 mg/kg	*0*	*0*	*0*	*0*		
chloracetamide 18 mg/kg	*30*	*33*	*75*	*83*		
chloracetamide 32 mg/kg	*40*	*40*	*100*	*100*		
chloracetamide 56 mg/kg	*40*	*40*	*100*	*100*		

The "No Observed Effect Concentration" (NOEC) is given as >1000 mg/kg on the basis that no significant mortalities were observed after 14 days exposure additionally no sub-lethal effects on weight or behaviour were observed at 1000 mg/kg.

Conclusion:

The 14 day LC50 of triticonazole to earthworms was > 1000 mg/kg.

The NOEC value was reported to be 1000 mg/kg.

Lührs U., 2002a – Acute Toxicity (14 days) of RPA 406341 to the Earthworm Eisenia fetida in Artificial Soil

Document number: C020497

Eisenia fetida in Artificial Soil, (Study AE 0540093, Report C020497)

Guideline: OECD Guidelines for Testing of Chemicals (1984), No. 207; ISO-Guideline 11268-1

GLP: Yes

Material and methods:

The purpose of this study was to determine the acute toxicity of RPA 406341 to the earthworm Eisenia fetida 7 and 14 days after exposure and to estimate the LC50 of RPA 406341. The concentrations of the test substance mixed into the artificial soil were 198, 296, 444, 667 and 1000 mg/kg. The control was treated with deionised water and quartz sand. Toxic standard: 2-Chloroacetamide. The following soil was used:

10 % Sphagnum-peat

20 % Kaolin clay

0.5 % CaCO3

69.5 % fine quartz sand

The pH of the soil ranged from 5.7 to 5.8. Water content at the start of the study was 35.0-36.2% (51.1-52.8 % WHC), and 35.0- 38.4% (51.1-56.1% WHC) at study termination. The test was carried out at 19-20°C and under continuous light regime.

LC_{50} and its 95% confidence limits at days 7 and 14 were not determined by a statistical analysis as no mortality was observed.

Data on earthworm body weight were tested for normality and homogeneity of variance using Kolmogoroff-Smirnov test ($\alpha = 0.05$) and Cochran test ($\alpha = 0.05$).

Because the body weight data did fulfil the criterion of homogeneity (Cochran test), the Dunnett test was used (multiple comparison, two-sided, $\alpha = 0.05$).

Findings:

After 14 days exposure no mortality was recorded in the control group and in the treatment groups up to 1000 mg/kg. There were no observed behaviour effects and no significant effect on earthworm weights up to 667 mg/kg. In the treated group with 1000 mg/kg the body weight decreased was significant compared to controls.

The toxicity of the positive control was within the expected range for the laboratory.

Table B.9.6.1-2: Earthworm body weight changes (mean of 4 replicates)

RPA 406341 (mg/kg)	*Test start*	*After 14 d*		
	mg/worm	*mg/worm*	*% difference*	*significance*
Control	*440.5*	*405.3*	*-7.9*	*-*
198	*436.2*	*387.8*	*-11.0*	*not signif.*
296	*446.2*	*401.2*	*-9.9*	*not signif.*
444	*438.6*	*396.2*	*-9.5*	*not signif.*
667	*465.3*	*404.6*	*-13.1*	*not signif.*
1000	*445.1*	*352.2*	*-20.7*	*signif. (Dunnett)*

Conclusions:

According to the results of this study the 14 day LC50 of RPA 406341 for earthworms was determined to be >1000 mg/kg artificial soil (dw). Due to significant body weight changes the LOEC was determined as 1000 mg/kg and the NOEC as 667 mg/kg artificial soil (dw).

Lührs U., 2001a – Acute Toxicity (14 days) of RPA 404766 to the Earthworm Eisenia fetida in Artificial Soil

Document number: C017900

Guideline: OECD Guidelines for Testing of Chemicals (1984), No. 207; ISO-Guideline 11268-1

GLP: Yes

Material and methods:

The purpose of this study was to determine the acute toxicity of RPA 404766 to the earthworm Eisenia fetida 7 and 14 days after exposure and to estimate the LC50 of RPA 404766. The concentrations of the test substance mixed into the artificial soil were 63, 125, 250, 500 and 1000 mg/kg. The control was treated with deionised water and quartz sand. Toxic standard: 2-Chloroacetamide. The following soil was used:

10 % Sphagnum-peat

20 % Kaolin clay

0.5 % CaCO3

69.5 % fine quartz sand

The pH of the soil ranged from 5.7 to 5.9. Water content at the start of the study was 32.1-33.6% (50.6-53.0 % WHC), and 32.9- 34.9% (51.9-55.0% WHC) at study termination. The test was carried out at 19-21°C and under continuous light regime.

LC_{50} and its 95% confidence limits at days 7 and 14 were not determined by a statistical analysis as no mortality was observed.

Data on earthworm body weight were tested for normality and homogeneity of variance using Kolmogoroff-Smirnov test ($\alpha = 0.05$) and Cochran test ($\alpha = 0.05$).

Because the body weight data did fulfil the criterion of homogeneity (Cochran test), the Dunnett test was used (multiple comparison, two-sided, $\alpha = 0.05$).

Findings:

After 14 days exposure no mortality was recorded in the control group and in the treatment groups up to 1000

mg/kg. There were no observed behaviour effects and no significant effect on earthworm weights up to 250 mg/kg. In the treated group with 500 and 1000 mg/kg the body weight decrease was significant compared to controls.

The toxicity of the positive control was within the expected range for the laboratory.

Table B.9.6.1-3: Earthworm body weight changes (mean of 4 replicates)

RPA 404766 (mg/kg)	*Test start*	*After 14 d*		
	mg/worm	*mg/worm*	*% difference*	*significance*
Control	*425.2*	*418.1*	*-1.6*	*-*
63	*390.0*	*385.1*	*-1.3*	*not signif.*
125	*440.2*	*409.00*	*-6.9*	*not signif.*
250	*418.0*	*392.8*	*-6.2*	*not signif.*
500	*406.2*	*372.3*	*-8.4*	*signif. (Dunnett)*
1000	*423.3*	*380.6*	*-10.0*	*signif. (Dunnett)*

Conclusions:

According to the results of this study the 14 day LC50 of RPA 404766 for earthworms was determined to be >1000 mg/kg artificial soil (dw). Due to significant body weight changes the LOEC was determined as 500 mg/kg and the NOEC as 250 mg/kg artificial soil (dw).

Lührs U., 2002b – Acute Toxicity (14 days) of RPA 407922 to the Earthworm Eisenia fetida in Artificial Soil

Document number: C021833

Guideline: OECD Guidelines for Testing of Chemicals (1984), No. 207; ISO-Guideline 11268-1

GLP: Yes

Material and methods:

The purpose of this study was to determine the acute toxicity of RPA 407922 to the earth worm Eisenia fetida 7 and 14 days after exposure and to estimate the LC50 of RPA 407922. The concentrations of the test substance mixed into the artificial soil were 95, 171, 309, 556 and 1000 mg/kg. The control was treated with deionised water and quartz sand. Toxic standard: 2-Chloroacetamide. The following soil was used:

10 % Sphagnum-peat

20 % Kaolin clay

0.5 % CaCO3

69.5 % fine quartz sand

The pH of the soil ranged from 6.0 to 6.1 (beginning of the study) and from 5.5-5.6 (study end). Water content at the start of the study was 31.3-33.3% (49.4-52.5 % WHC), and 32.1- 36.1% (50.6-57% WHC) at study termination. The test was carried out at 19-20°C and under continuous light regime.

LC50 and its 95% confidence limits at days 7 and 14 were not determined by a statistical analysis as no mortality was observed.

Data on earthworm body weight were tested for normality and homogeneity of variance using Kolmogoroff-

Smirnov test ($\alpha = 0.05$) and Cochran test ($\alpha = 0.05$).

Because the body weight data did fulfil the criterion of homogeneity (Cochran test), the Dunnett test was used (multiple comparison, two-sided, $\alpha = 0.05$).

Findings:

After 14 days exposure no mortality was recorded in the control group and in the treatment groups up to 1000 mg/kg. There were no observed behaviour effects and no significant effect on earthworm weights up to 556 mg/kg. In the treated group with 1000 mg/kg the body weight decrease was significant compared to controls.

The toxicity of the positive control was within the expected range for the laboratory.

Table B.9.6.1-4: Earthworm body weight changes (mean of 4 replicates)

RPA 407922 (mg/kg)	*Test start*	*After 14 d*		
	mg/worm	*mg/worm*	*% difference*	*significance*
Control	*425.2*	*418.1*	*-6.2*	*-*
95	*390.0*	*385.1*	*-7.3*	*not signif.*
171	*440.2*	*409.00*	*-5.5*	*not signif.*
309	*418.0*	*392.8*	*-8.1*	*not signif.*
556	*406.2*	*372.3*	*-9.0*	*not signif.*
1000	*423.3*	*380.6*	*-14.9*	*signif. (Dunnett)*

Conclusions:

According to the results of this study the 14 day LC50 of RPA 404766 for earthworms was determined to be >1000 mg/kg artificial soil (dw). Due to significant body weight changes the LOEC was determined as 1000 mg/kg and the NOEC as 556 mg/kg artificial soil (dw).

B.9.4.2. Earthworm – sub-lethal effects

In the first EU approval of triticonazole, the chronic toxicity of triticonazole for earthworms was addressed with an earthworm reproduction study. For the current submission additional earthworm reproduction studies conducted with the major soil metabolites were provided. The studies were evaluated according to the current valid testing guidelines and the study summaries are given below.

Active substance:

Reference:	**Effects of triticonazole on reproduction and growth of earthworms *Eisenia fetida* (Savigny 1826) in artificial soil**
Author(s), year:	Lührs, U., 1999a
Report/Doc. number:	R006093
Guideline(s):	BBA 1994 part VI, 2-2; ISO-Guideline 11268-2
GLP:	Yes
Deviations:	Please refer to the commenting box below
Validity:	Acceptable

Material and methods:

Test substance:	Triticonazole, batch no.: 9802018, Purity: 965 g/kg according to certificate of analysis)
Test species:	Earthworm (Annelida: Oligochaeta), *Eisenia fetida* (Savigny 1862); commercial breeder Kraut & Rüben
Number of organisms:	4 replicates per treatment group and control group, each with 10 individuals
Weight, age:	350 – 550 mg/worm, weight range between test individuals not differing for 200 mg within this range; adults, approx. 8 month, with clitellum, age range between test individuals not differing for > 4 weeks
Acclimatisation:	2 days, in artificial soil, under test conditions
Type of test, duration:	Laboratory sub-lethal test, 8 weeks (4 weeks adult mortality, 4 weeks juvenile development)
Applied concentrations:	31.25, 62.50, 125, 250, 500 mg ai/kg soil dw, incorporated into the soil
Control:	Deionized water
Solvent:	None
Toxic standard:	Derosal SC 360 g/L, containing 32.8%, tested ate 7 mg/kg artificial soil
Test substrate:	Artificial soil, 10 % sphagnum peat, 20 % kaolin clay, approx. 69.6 % fine quartz sand, approx. 0.4% chalk ($CaCO_3$)
Substrate/test vessel:	500 g dry weight/test container
Temperature:	17 – 23 °C
Light regime:	16 hours light, 8 hours dark; light intensity: 480-780

Water content:	Test start: 27.5-28.8% (equivalent to 56.0-58.7% of WHC) Test end: 26.7-35.5% (equivalent of 54.5-72.3 % of WHC)
pH:	Test start: 6.0 Test end: 5.7 – 5.8
Feeding:	Finely ground cattle manure; 10 g/kg mixed into soil at start of the study, 5 g/container scattered on the soil surface at day 1 after application, 5 g/container (depended on the amount consumed) was added weekly during the first 4 weeks.
Test parameters:	Temperature and air humidity were recorded continuously during the whole test period. The water content and the pH of the artificial soil were determined at the start of the test, with 1 sample in each treatment group and at the end of the test, with measurement in 2 containers per test concentration and control. The water content was checked once per week. Mortality of adults (assessed after 28 days), mean body weight of adults (measured at day 0 and after 28 days), morphological and behavioural changes of adults (observed at day 28), number of juvenile earthworms (counted after 8 weeks) and condition and behaviour of juveniles (observed after 8 weeks). Approximated mean food consumption per container for each treatment group.
Statistics:	The endpoints were mortality, change of biomass (difference in fresh weight of surviving worms between test start and four weeks after treatment) and reproduction (the number of juveniles present). The arithmetic mean and the standard deviation per treatment and per control for reproduction and biomass were calculated. For statistical analysis Dunnett - test ($\alpha = 0.05$) was used.
Findings:	No behavioural abnormalities were observed. The amount of food added to the test containers was 19 g for the control and between 18 and 20 g for the test concentrations

Table 9.4-1: Effects of triticonazole on mortality and reproduction of *Eisenia fetida* in a sub-chronic test

Treatment group [mg ai/kg soil d.w.]	Mortality of adult earthworms after 4 weeks [%] (± SD)	Statistical evaluation	Body weight increase per adult earthworm between day 0 and after 4 weeks [%] (± SD)	Statistical evaluation	Mean number of young earthworms per container (± SD)	Statistical evaluation
Control	2.5 (5.0)	-	2.0 (0.5)	-	188 (38)	-
31.25	7.5 (5.0)	n.s.	5.7 (5.8)	n.s.	179 (40)	n.s.
62.5	10.0 (8.2)	n.s.	14.3 (3.1)	n.s.	246 (51)	n.s.
125	5.0 (5.8)	n.s.	17.0 (8.2)	n.s.	227 (62)	n.s.
250	5.0 (5.8)	n.s.	15.1(12.7)	n.s.	164 (97)	n.s.
500	7.5 (9.6)	n.s.	12.7 (3.8)	n.s.	146 (97)	n.s.
Toxic standard	10.0 (14.1)	n.s.	1.0 (7.0)	n.s.	16 (5)	*

SD…Standard Deviation
*statistically significantly different compared to control
n.s. not significantly different compared to control

In the positive control (Carbendazim) the number of juveniles was reduced by 91.7% at concentrations of 7 mg prod./kg soil dw (mean number of juveniles = 16) after 8 weeks of test duration when compared to the control (mean number of juveniles = 188).

Conclusion: NOEC = 500 mg test item/kg soil dw (adult mortality, body weight, reproduction)
LOEC > 500 mg test item/kg soil dw
EC_{50} > 500 mg test item/kg soil dw

Comment RMS: The study was evaluated following the recommendations of the currently valid test guideline OECD 222 (2016)

Check of validity criteria:

- Each replicate (containing 10 adults) of the control should have produced ≥ 30 juveniles by the end of the test. In the current test number of juveniles per replicate was 188 ± 38. Fulfilled.
- The coefficient of variation of reproduction in the control should be ≤ 30 %. In the current study the coefficient of variation was 10.23%. Fulfilled.
- Adult mortality over the initial 4 weeks of the test in the control should be ≤ 10 %. In the current study the mortality was 2.5 % ± 5 %. Fulfilled.

In addition, the following points deviated from the test guideline or were not reported in detail:

- According to OECD 222 for substances insoluble in water and organic test substance, 10 g of finely industrial quartz sand are mixed with the needed quantity of test substance. In the current test 20 g quartz sand was used. Furthermore the test item is soluble in organic solvent.

	- According to OECD 222 test containers should be left uncovered for a period of one hour. In the current study the containers were left uncovered for half an hour. - According to OECD 222 the test temperature should be 20 ± 2 °C. In the current test temperature was > 22 °C (max. 23 °C) for 5 hours and < 18 °C (min 17°C) for 10 hours. - According to OECD 222 food is first provided one day after adding the worms and applying the test chemical to the soil (5 g/kg). In the current study 10 g/kg food was mixed into to the soil at the start of the test. **Acceptability of the analytical methods used in the test:** No information regarding the analytical methods was reported. **Endpoints:** In the study report a NOEC of 500 mg test item/kg soil dw based on adult mortality, body weight and reproduction is proposed. However, at 500 mg/kg soil dw 22.1% effects on reproduction were observed. Therefore the RMS recommends following endpoints NOEC = 250 mg test item/kg soil dw. EC_{50} > 500 mg test item/kg soil dw According to ToxRat® 3.1.0. EC_{10} values could not be calculated. **Conclusion of the RMS:** The treatment groups 62.5 and 125 mg ai/kg soil dw showed a higher number of juveniles than in the control group (246 and 227 in comparison to 188 juveniles in the control). However, the guideline recommends a one-sided t-test, not considering an increase in reproduction as an adverse effect. Based on the evaluation of the study the chronic earthworm toxicity test is considered valid.

Metabolites:

Reference:	**Sublethal toxicity of Reg. No. 5079285 (metabolite of BAS 595 F, triticonazole) to the earthworm *Eisenia fetida* in artificial soil**
Author(s), year:	Friedrich, S., 2013a
Report/Doc. number:	BASF DocID 2014/1000026
Guideline(s):	OECD 222 (2004)
GLP:	Yes
Deviations:	Please refer to the commenting box below
Validity:	Acceptable

Material and methods:

Test substance:	Reg. No. 5079285 (RPA 404766, metabolite of triticonazole), batch no.: L67-148, analysed purity: 99.3% (tolerance ± 1.0%)
Test species:	Earthworm *Eisenia fetida* (Savigny, 1826), subspecies (Bouché, 1972), reared in test facility (original breeding purchased from W. Neudorff GmbH KG)
Number of organisms:	8 replicates for the control group, 4 replicates per treatment group, each with 10 individuals.
Weight, age:	Mean: 302 - 450 mg/worm, adults with clitellum, approximately 3 months
Acclimatisation:	Approx. 24 hours, in the artificial substrate with food
Type of test, duration:	Laboratory sub-lethal test, 8 weeks
Applied concentrations:	Control, 15.63, 31.25, 62.5, 125, 250 mg test item/ kg soil dw incorporated into soil
Solvent:	None
Toxic standard:	Nutdazim 50 FLOW (Carbendazim, SC 500), tested in a separate study at concentrations of 5 and 10 mg prod./kg soil dw
Test substrate:	Artificial soil, 10 % sphagnum peat, 20 % kaolin clay, 69.5 % industrial quartz sand, 0.5% calcium carbonate,
Substrate/test vessel:	600 g dry weight/test container
Temperature:	18.1 – 21.9 °C
Light regime:	16 hours light, 8 hours dark; light intensity: 540 lux
Water content:	Test start: 34.9-35.0 % (equivalent to 54.7-54.9 % of WHC) Test end: 34.4-34.6% (equivalent of 53.9-54.2 % of WHC)
pH:	Test start: 6.00 – 6.06 Test end: 5.70 – 5.74
Feeding:	Air-dried and finely ground horse manure; feeding interval was weekly during the first 4 weeks, weekly amount of manure (5 g) depended on the feeding activity.
Test parameters:	Temperature was recorded continuously during the whole test period. The moisture content and the pH of the artificial soil were determined at the start and

the end of the test.

Mortality of adults (assessed after 28 days), mean body weight of adults (measured at day 0 and after 28 days), morphological and behavioural changes of adults (observed at day 28), number of juvenile earthworms (counted after 8 weeks) and condition and behaviour of juveniles (observed after 8 weeks)

Statistics: The endpoints were mortality, change of biomass (difference in fresh weight of surviving worms between test start and four weeks after treatment) and reproduction (the number of juveniles present). The arithmetic mean and the standard deviation per treatment and per control for reproduction and biomass were calculated.

The statistical analysis was performed with the software ToxRat Professional 2.10.06 (RATTE 2010). Shapiro-Wilk´s Test and Levene´s test were used, respectively, to test the data for normality and homogeneity of variance. Fisher's Exact Binomial Test with Bonferroni Correction and Williams-t-test were used to compare the control with the independent test item group. For statistical evaluation of the biomass change, the changed mean fresh weight of surviving worms per replicate was used.

Findings: No effects on behaviour (including feeding activity) of the worms were observed during the test.

Table 9.4-2: Effects of Reg. No. 5079285 on mortality and reproduction of *Eisenia fetida* in a sub-chronic test

Treatment group [mg ai/kg soil d.w.]	Mortality of adult earthworms after 4 weeks [%]	Statistical evaluation	Body weight differences per adult earthworm after 4 weeks relative to initial fresh weight [%]	Statistical evaluation	Reproduction (number of young earthworms per container [% of control]	Statistical evaluation
Control	2.5	-	34.9	-	100	-
15.63	2.5	n.s.	36.8	n.s.	110.6	n.s.
31.25	0.0	n.s.	33.4	n.s.	95.3	n.s.
62.5	0.0	n.s.	36.1	n.s.	104.0	n.s.
125	2.5	n.s.	36.7	n.s.	98.6	n.s.
250	0.0	n.s.	31.9	n.s.	92.0	n.s.

*statistically significant different compared to control
n.s. not statistically significant different compared to control

In the positive control (Carbendazim) the number of juveniles was reduced by 72.7 and 98.8 % at concentrations of 5 and 10 mg prod./kg soil dw (mean number of juveniles = 23 and 1) after 8 weeks of test duration when compared to the control (mean number of juveniles = 84).

Conclusion:	NOEC = 250 mg test item/kg soil dw (adult mortality, body weight, reproduction) EC_{50} > 250 mg test item/kg soil dw

Comment RMS:	The study was evaluated following the recommendations of the currently valid test guideline OECD 222 (2016) **Check of validity criteria:** - Each replicate (containing 10 adults) of the control should have produced ≥ 30 juveniles by the end of the test. In the current test number of juveniles per replicate was 106 ± 20.2. Fulfilled. - The coefficient of variation of reproduction in the control should be ≤ 30 %. In the current study the coefficient of variation was 19.0%. Fulfilled. - Adult mortality over the initial 4 weeks of the test in the control should be ≤ 10 %. In the current study the mortality was 2.5 %. Fulfilled **In addition, the following points deviated from the test guideline or were not reported in detail:** - According to OECD 222 test containers should be left uncovered for a period of one hour. In the current study the containers were left uncovered for half an hour. - According to OECD 222 a combined approach allows for determination of both the NOEC and EC_x. Eight treatment concentrations in a geometric series should be used. Four replicates for each treatment plus eight controls are recommended. The concentrations should be spaced by a factor not exceeding 1.8. In the current study the spacing factor was 2. **Acceptability of the analytical methods used in the test:** No information regarding the analytical methods was reported. **Endpoints:** The RMS agrees on the endpoints given in the study report. NOEC = 250 mg test item/kg soil dw (adult mortality, body weight, reproduction) EC_{50} > 250 mg test item/kg soil dw According to ToxRat® 3.1.0. EC_{10} values could not be calculated. **Conclusion of the RMS:** Based on the evaluation of the study the chronic earthworm toxicity test is considered valid.

Reference:	**Sublethal toxicity of Reg. No. 5079288 (metabolite of BAS 595 F, triticonazole) to the earthworm *Eisenia fetida* in artificial soil**
Author(s), year:	Friedrich, S., 2013b
Report/Doc. number:	BASF DocID 2014/1000027
Guideline(s):	OECD 222 (2004)
GLP:	Yes
Deviations:	Please refer to the commenting box below
Validity:	Acceptable

Material and methods:

Test substance:	Reg. No. 5079288 (RPA 407922, metabolite of triticonazole), batch no.: 33484-39, analysed Purity: 99.5% (tolerance ± 1)
Test species:	Earthworm *Eisenia fetida* (Savigny, 1826), subspecies (Bouché, 1972), reared in test facility (original breeding purchased from W. Neudorff GmbH KG)
Number of organisms:	8 replicates for the control group and 4 replicates per treatment group, each with 10 individuals.
Weight, age:	Mean: 284 – 468 mg/worm, adults with clitellum, approximately 3 months
Acclimatisation:	Approx. 24 hours, in the artificial substrate with food
Type of test, duration:	Laboratory sub-lethal test, 8 weeks (4 weeks adult mortality, 4 weeks juvenile development)
Applied concentrations:	Control, 15.63, 31.25, 62.5, 125 and 250 mg test item/kg soil dw, incorporated into the soil
Solvent:	None
Toxic standard:	Nutdazim 50 FLOW (Carbendazim, SC 500), tested in a separate study at concentrations of 5 and 10 mg prod./kg soil dw
Test substrate:	Artificial soil, 10 % sphagnum peat, 20 % kaolin clay, 69.5 % industrial quartz sand, 0.5% calcium carbonate,
Substrate/test vessel:	600 g dry weight/test container
Temperature:	18.6 – 21.9 °C
Light regime:	16 hours light, 8 hours dark; light intensity: 520 lux
Water content:	Test start: 34.9 – 35.1% (equivalent to 54.7 – 55.0% of WHC) Test end: 34.5 – 34.8% (equivalent of 54.1 – 54.5% of WHC)
pH:	Test start: 6.12 – 6.16 Test end: 5.71 – 5.77
Feeding:	Air-dried and finely ground horse manure; feeding interval was weekly during the first 4 weeks, weekly amount of manure (5 g) depended on the feeding activity.
Test parameters:	Temperature was recorded continuously during the whole test period. The moisture content and the pH of the artificial soil were determined at the start and the end of the test.

	Mortality of adults (assessed after 28 days), mean body weight of adults (measured at day 0 and after 28 days), morphological and behavioural changes of adults (observed at day 28), number of juvenile earthworms (counted after 8 weeks) and condition and behaviour of juveniles (observed after 8 weeks)
Statistics:	The endpoints were mortality, change of biomass (difference in fresh weight of surviving worms between test start and four weeks after treatment) and reproduction (the number of juveniles present). The arithmetic mean and the standard deviation per treatment and per control for reproduction and biomass were calculated. The statistical analysis was performed with the software ToxRat Professional 2.10.06 (RATTE 2010). The EC50 (number of juveniles) was calculated by Probit analysis using the maximum likelihood method (Finney 1971). Shapiro-Wilk´s Test and Levene´s test were used, respectively, to test the data for normality and homogeneity of variance. Fisher's Exact Binomial Test with Bonferroni Correction and Williams-t-test were used to compare the control with the independent test item group. For statistical evaluation of the biomass change, the changed mean fresh weight of surviving worms per replicate was used.
Findings:	No effects on behaviour (including feeding activity) of the worms were observed during the test.

Table 9.4-3: Effects of Reg. No. 5079288 on mortality and reproduction of *Eisenia fetida* in a sub-chronic test

Treatment group [mg ai/kg soil d.w.]	Mortality of adult earthworms after 4 weeks [%]	Statistical evaluation	Body weight differences per adult earthworm after 4 weeks relative to initial fresh weight [%]	Statistical evaluation	Reproduction (number of young earthworms per container [% of control]	Statistical evaluation
Control	0.0	-	41.6	-	100	-
15.63	0.0	n.s.	40.8	n.s.	95.4	n.s.
31.25	0.0	n.s.	42.5	n.s.	99.1	n.s.
62.5	5.0	n.s.	41.1	n.s.	95.0	n.s.
125	0.0	n.s.	44.8	n.s.	86.9	n.s.
250	5.0	n.s.	40.1	n.s.	73.4	*

*statistically significant different compared to control
n.s. not statistically significantl different compared to control

In the positive control (Carbendazim) the number of juveniles was reduced by 72.7 and 98.8 % at concentrations of 5 and 10 mg prod./kg soil dw (mean number of juveniles = 23 and 1) after 8 weeks of test duration when compared to the control (mean number of juveniles = 84).

Conclusion:	NOEC = 250 mg test item/kg soil dw (adult mortality, body weight) NOEC = 125 mg test item/kg soil dw (reproduction) EC_{50} > 250 mg test item/kg soil dw

Comment RMS:	The study was evaluated following the recommendations of the currently valid test guideline OECD 222 (2016) **Check of validity criteria:** - Each replicate (containing 10 adults) of the control should have produced ≥ 30 juveniles by the end of the test. In the current test number of juveniles per replicate was 122.9 ± 19.6. Fulfilled. - The coefficient of variation of reproduction in the control should be ≤ 30 %. In the current study the coefficient of variation was 16.0%. Fulfilled. - Adult mortality over the initial 4 weeks of the test in the control should be ≤ 10 %. In the current study the mortality was 0.0 %. **In addition, the following points deviated from the test guideline or were not reported in detail:** - According to OECD 222 test containers should be left uncovered for a period of one hour. In the current study the containers were left uncovered for half an hour. - According to OECD 222 a combined approach allows for determination of both the NOEC and EC_x. Eight treatment concentrations in a geometric series should be used. Four replicates for each treatment plus eight controls are recommended. The concentrations should be spaced by a factor not exceeding 1.8. In the current study the spacing factor was 2. **Acceptability of the analytical methods used in the test:** No information regarding the analytical methods was reported. **Endpoints:** The RMS agrees on the endpoints given in the study report. NOEC = 125 mg test item/kg soil dw (reproduction) EC_{50} > 250 mg test item/kg soil dw According to ToxRat® 3.1.0. EC_{10} values could not be calculated. **Conclusion of the RMS:** Based on the evaluation of the study the chronic earthworm toxicity test is considered valid.

Reference:	**Effects of Reg.No. 5059144 (RPA 406341, metabolite of BAS 595 F) on growth and reproduction of earthworms (*Eisenia fetida*) in artificial soil with 5% peat**
Author(s), year:	Wolf, A., 2006a
Report/Doc. number:	BASF DocID 2006/1030247
Guideline(s):	OECD 222 (2004)
GLP:	Yes
Deviations:	Please refer to the commenting box below
Validity:	Acceptable

Material and methods:

Test substance:	Reg. No. 5059144; batch no.: BESS0541, purity: 99.0%
Test species:	Earthworm *Eisenia fetida*; in-house culture.
Number of organisms:	8 replicates for the control group and 4 replicates per treatment group, each with 10 individuals.
Weight, age:	Mean: 302 – 481 mg/worm, adults < 1 year
Acclimatisation:	Approx. 24 hours, in the artificial substrate with food
Type of test, duration:	Laboratory sub-lethal test, 8 weeks (4 weeks adult mortality, 4 weeks juvenile development)
Applied concentrations:	Control, 1 and 10 mg test item/kg soil dw, incorporated into the soil
Solvent:	Acetone
Toxic standard:	Benlate (BAS 321 00 F), 50.0 % Benomyl (nominal) tested at 5 mg prod./kg soil dw
Test substrate:	Artificial soil, 5 % sphagnum peat, 20 % kaolin clay, 75 % industrial quartz sand, calcium carbonate,
Substrate/test vessel:	600 g dry weight/test container
Temperature:	19-21 °C
Light regime:	16 hours light, 8 hours dark; light intensity: 400-700 lux
Water content:	About 60% of the maximum holding capacity Not stated in more detail in the study report.
pH:	Test start: 5.74 – 5.85 Test end: 6.18 – 6.29
Feeding:	Powdered cow manure; feeding interval was weekly during the first 4 weeks, weekly amount of manure (5 g) depended on the feeding activity.
Test parameters:	Temperature was recorded continuously during the whole test period. The pH of the artificial soil was determined at the start and the end of the test. Mortality of adults (assessed after 28 days), mean body weight of adults (measured at day 0 and after 28 days and behavioural changes of adults (observed at day 28), number of juvenile earthworms (counted after 8 weeks) and condition and behaviour of juveniles (observed after 8 weeks)

Statistics: The endpoints were mortality, change of biomass (difference in fresh weight of surviving worms between test start and four weeks after treatment) and reproduction (the number of juveniles present).
The statistical analysis was performed with the software TOXSTAT 3.5. Shapiro-Wilk´s Test and Bartlett's test were used, respectively, to test the data for normality and homogeneity of variance. Statistical analysis on worm biomass and number of offspring was evaluated by analysis of variance and Bonferroni t-test.

Findings: No effects on behaviour of the worms were observed during the test.

Table 9.4-4: Effects of Reg. No. 5059144 on mortality and reproduction of *Eisenia fetida* in a sub-chronic test

Treatment group [mg ai/kg soil d.w.]	Mortality of adult earthworms after 4 weeks [%]	Statistical evaluation	Body weight differences per adult earthworm after 4 weeks relative to initial fresh weight [%] (SD)	Statistical evaluation	Reproduction (mean number of young earthworms per container) (SD)	Statistical evaluation
Control	0.0	-	59.96 (7.93)	-	105 (13.794)	-
1.	0.0	n.s.	61.33 (1.86)	n.s.	91.75 (3.862)	n.s.
10	0.0	n.s.	57.87 (8.58)	n.s.	112 (23.903)	n.s.
Toxic reference	0.0	n.s.	22.61 (9.23)	*	6.5 (4.933)	*

SD standard deviation
*statistically significant different compared to control
n.s. not statistically significantl different compared to control

Conclusion: 28 day NOEC = 10 mg test item/kg soil dw (adult mortality, body weight)
56 day NOEC = 10 mg test item/kg soil dw (reproduction)
LC_{50} > 10 mg test item/kg soil dw

Comment RMS: The study was evaluated following the recommendations of the currently valid test guideline OECD 222 (2016)

Check of validity criteria:

- Each replicate (containing 10 adults) of the control should have produced ≥ 30 juveniles by the end of the test. In the current test number of juveniles per replicate was 105 ± 13.794. Fulfilled.
- The coefficient of variation of reproduction in the control should be ≤ 30 %. In the current study the coefficient of variation was 13.138%. Fulfilled
- Adult mortality over the initial 4 weeks of the test in the control should be ≤ 10 %. In the current study the mortality was 0.0 %.

In addition, the following points deviated from the test guideline or were not reported in detail:

- According to OECD 222 for substances insoluble in water The test chemical is dissolved in a small volume of a suitable organic solvent (e.g. acetone) and then sprayed onto, or mixed into, a small quantity of fine quartz sand. The solvent is then removed by evaporation in a fume hood for at least a few minutes. In the current study acetone was used as solvent. However it is not reported that the solvent was removed.
- According to OECD 222 if solvents are used to aid treatment of the soil with the test chemical an appropriate solvent control must be included in the test design. In the current study acetone was used as solvent, but no solvent control was tested.

Acceptability of the analytical methods used in the test: No information regarding the analytical methods was reported.

Endpoints:

The RMS agrees on the endpoints given in the study report.

28 day NOEC = 10 mg test item/kg soil dw (adult mortality, body weight)

56 day NOEC = 10 mg test item/kg soil dw (reproduction)

EC_{50} > 10 mg test item/kg soil dw

According to ToxRat® 3.1.0. EC_{10} values could not be calculated.

Conclusion of the RMS: No solvent control was tested. However as no effects were seen, neither in the control nor in the treatment groups, it is considered acceptable.

Based on the evaluation of the study the chronic earthworm toxicity test is considered valid.

B.9.4.3. Effects on non-target soil meso- and macrofauna (other than earthworms)

According to the data requirements on active substances (Regulation 283/2013) the risk to soil dwelling organisms has to be addressed (1) if a risk to non-target arthropods was identified or (2) if the product is applied to the bare soil (pre-emergence).
Under consideration of the intended uses as seed treatment the risk to soil meso- and macrofauna from exposure to the active substance and its major soil metabolites has to be addressed. Hence, laboratory studies with the soil organisms *Folsomia candida* and *Hypoaspis aculeifer* were submitted.

Active substance:

Reference:	**Effects of BAS 595 F (triticonazole) on the reproduction of the collembolan *Folsomia candida***
Author(s), year:	Friedrich, S., 2013c
Report/Doc. number:	BASF DocID 2014/1000021
Guideline(s):	OECD 232 (2009), ISO 11267 (1999)
GLP:	Yes
Deviations:	Please refer to the commenting box below
Validity:	Acceptable

Material and methods:

Test substance:	BASF 595 F (triticonazole); batch no.: COD-001440, Purity: 91.3% w/w analysed (tolerance ± 1%)
Test species:	Collembola *Folsomia candida* (Willem); in-house culture (originally purchased from "Biologische Bundesanstalt"), Berlin-Dahlem
Number of organisms:	8 replicates per control and solvent control, 4 replicates for the 5 treatment groups, each with 10 individuals. 2 additional replicates per treatment control and solvent control for measurement purposes.
Life stage, age:	Juveniles 9-12 days old
Type of test, duration:	Chronic laboratory dose-response test, 28 days
Applied concentrations:	Control, solvent control, 62.5, 125, 250, 500, 1000 mg ai/kg soil dry weight (based on analysed purity) incorporated into the soil
Solvent:	Acetone
Toxic standard:	Boric acid, Purity: 100% (analysed), the effects of the reference item were investigated in a separate study at concentrations of 44, 67, 100, 150 and 225 mg/kg soil dry weight.
Test substrate:	Artificial soil, 5 % peat, 20 % kaolin clay, 74.7 % industrial quartz sand, 0.3% calcium carbonate;

Substrate/test vessel:	30 g wet weight/test container
Temperature:	18.3 – 21.9 °C
Light regime:	16 hours light, 8 hours dark; light intensity: 520 lux
Water content:	Test start: 56.8 – 57.% of WHC Test end: 55.9-56.4% of WHC
pH:	Test start: 6.06-6.14 Test end: 5.75-5.8
Feeding:	Approximately 2 mg dry yeast at test start and on day 14.
Test parameters:	pH and water content were determined at test start and test end. Water content maintenance was checked weekly after application. Mortality of adults, reproductive output (number of juvenile Collembola) were assessed after 28 days.
Statistics:	Fisher`s Exact Binomial Test with Bonferroni correction for mortality ($\alpha = 0.05$, one-sided greater), Williams-t-test for reproduction ($\alpha = 0.05$, one-sided smaller). The software used to perform the statistical analysis was ToxRat Professional, Version 2.10.06 (RATTE 2010)
Findings:	Differences between the behaviour of the collembolans in the control groups and the test item treatment groups could not be observed.

Table 9.4-5: Effects on mortality and reproduction of *Folsomia candida* in a chronic test

Treatment group [mg ai/kg soil d.w.]	Mean number of surviving parental collembolans after 28 d (± SD)	Mortality of adult Collembola after 28 d [%]	Mean number of juveniles after 28 d (± SD)	Reproduction [% of control]
Control	9.9 (0.4)	1.3	1303 (111.5)	-
Solvent control	9.8 (0.5)	2.5	1276 (147.0)	100
62.5	9.8 (0.5)	2.5	1271 (179.9)	100
125	9.8 (0.5)	2.5	1312 (143.8)	103
250	8.8 (0.5)	12.5	1227 (136.8)	96
500	8.3 (0.5)	17.5*	1282 (231.3)	100
1000	6.3 (0.5)	37.5*	1003* (203.0)	79

SD…Standard Deviation
*statistically significant different compared to control

In the most recent study the LC_{50} and EC_{50} for the reference item boric acid was determined to be 108 mg test item/kg soil dw and 192 mg test item/kg soil dw, respectively. The NOEC for mortality and for reproduction was determined to be 100 and 44 mg test item/kg soil dw, respectively.

Conclusion:	NOEC = 250 mg test item/kg soil dw LC_{50} (mortality) > 1000 mg test item/kg soil dw

Comment RMS:	The study was evaluated following the recommendations of the currently valid test guideline OECD 232 (2016). **Check of validity criteria:** - Mean adult mortality in the controls should not exceed 20% at the end of the test. In the current study mortality was 1.3% and 2.5% in the control and solvent control, respectively. Fulfilled. - The mean number of juveniles per vessel in the controls should be at least 100 at the end of the test. In the current study the mean number of juveniles per vessel was 1303 and 1276 in the control and solvent control, respectively. Fulfilled. - The coefficient of variation calculated for the number of juveniles in the controls should be less than 30% at the end of the definitive test. In the current study the coefficient of variation was 9.8 % for the pooled controls. Fulfilled. **In addition, the following points deviated from the test guideline or were not reported in detail:** - According to OECD 232 for determination of the NOEC/LOEC, at least five concentrations in a geometric series should be tested. Four replicates for each test concentration treatment plus eight controls are recommended. The concentrations should be spaced by a factor not exceeding 1.8. A combined approach allows for determination of both the NOEC/LOEC and EC_x. For this combined approach, eight treatment concentrations in a geometric series should be used. Four replicates for each treatment plus eight controls are recommended. The concentrations should be spaced by a factor not exceeding 1.8. In the current study 5 concentrations with each 4 replicates were tested with a spacing factor of 2. **Acceptability of the analytical methods used in the test:** No information regarding the analytical methods was reported. **Endpoints:** The endpoints were recalculated by the RMS via ToxRat® 3.1.0 as follows, identifying significant effects on survival at 250 mg ai/kg soil dw: 28 day NOEC = 125 mg ai/kg soil dw 28 day LC_{50} > 1000 mg ai/kg soil dw According to ToxRat® 3.1.0. EC_{10} values could not be calculated. **Conclusion of the RMS:** Based on the evaluation of the study the chronic collembolan toxicity test is considered valid.

Reference:	**Effects of BAS 595 F (triticonazole) on the reproduction of the predatory mite *Hypoaspis aculeifer***
Author(s), year:	Schulz, L., 2014a
Report/Doc. number:	BASF DocID 2014/1000022
Guideline(s):	OECD 226 (2008)
GLP:	Yes
Deviations:	Please refer to the commenting box below
Validity:	Acceptable

Material and methods:

Test substance:	BAS 595 F, triticonazole; batch no. COD-001440, analysed purity: 91.3% (tolerance ± 1.0%)
Test species:	Predatory mites, *Hypoaspis aculeifer* (CANESTRINI); in-house culture (originally purchased from "Katz Biotech AG"
Number of organisms:	8 replicates per control and solvent control and 4 replicates per treatment group, 2 additional replicates per treatment and control to check the pH and water content of the test substrate after 14 days, each with 10 individuals.
Life stage:	Adult females
Type of test, duration:	Laboratory sub-lethal test, 14 days
Applied concentrations:	Control, solvent control, 62.5, 125, 250, 500, 1000 mg test item/kg soil dw incorporated into the soil
Solvent:	Acetone
Toxic standard:	Dimethoate EC 400, tested at concentrations 4.10, 5.12, 6.40, 8:00 and 10.00 mg ai/kg soil dw in a separate study.
Test substrate:	Artificial soil, 5 % sphagnum peat, 20 % kaolin clay, 74.6 % industrial quartz sand, 0.1% calcium carbonate
Substrate/test vessel:	20 g dry weight/test container
Temperature:	19.5 – 21.2°C
Light regime:	16 hours light, 8 hours dark; light intensity: 511 lux
Water content:	Test start: 22.7 – 23.81% (equivalent to 56.67 – 59.43% of WHC) Test end: 21.46 – 23.08% (equivalent of 53.56 – 57.61% of WHC)
pH:	Test start: 5.7– 5.8 Test end: 5.8 – 5.9
Feeding:	Before and during the test predatory mites were fed with cheese mites (*Tyrophagus putrescentiae*) twice to three times a week.
Test parameters:	pH and water content were determined at test start and test end. Mortality of adults, differences in morphology, behavioural effects and number of juveniles were assessed after 14 days.
Statistics:	The statistical analysis was performerd with ToxRat Professional, Version 2.10.05

(Ratte 2010)

Findings:

Biological effects: No differences in behaviour and morphology of the mites between the test item groups and the control were observed.

Table 9.4-6: Effects on mortality and reproduction of *Hypoaspis aculeifer* in a sub-chronic test

Treatment group [mg product/kg soil d.w.]	Mean number of surviving adult mites after 14 d (± SD)	Mortality of adult mites after 14 d [%]	Statistical evaluation	Mean number of juveniles after 14 d (± SD)	Reproduction [% of control]	Statistical evaluation
Control	9.8 (0.5)	2.5	n.s.	249.3 (16.0)	-	n.s.
Solvent control	9.6 (0.5)	3.8	-	246.9 (61.3)	100	-
62.5	9.8 (0.5)	2.5	n.s.	270.3 (26.7)	109	n.s.
125	9.5 (0.6)	5.0	n.s.	276.0 (40.3)	112	n.s.
250	10.0 (0.0)	0.0	n.s.	256.0 (18.5)	104	n.s.
500	9.8 (0.5)	2.5	n.s.	249.8 (28.9)	101	n.s.
1000	9.8 (0.5)	2.5	n.s.	203.8 (23.4)	83	n.s.

SD…Standard Deviation
n.s.…not statistically significant
Dimethoate EC 400 showed an EC_{50} of 6.64 mg ai/kg soil dw.

Conclusion: 14 day LC_{50} / EC_{50} (mortality, reproduction) > 1000 mg test item/kg soil dw
14 day NOEC (mortality, reproduction) = 1000 mg test item/kg soil dw

Comment RMS: The study was evaluated following the recommendations of the currently valid test guideline OECD 226 (2016).

Check of validity criteria:

- Mean adult mortality of the females in the controls should not exceed 20% at the end of the test. In the current study mortality was 2.5 % and 3.8 in the control and solvent control, respectively. Fulfilled.
- The mean number of juveniles per replicate in the controls should be at least 50 at the end of the test. In the current study the mean number of juveniles per vessel was 249.3 and 246.9 in the control and solvent control, respectively. Fulfilled.
- The coefficient of variation calculated for the number of juvenile mites in the controls should not be higher than 30% at the end of the definitive test. In the current study the coefficient of variation was 17.5% for the pooled control. Fulfilled.

In addition, the following points deviated from the test guideline or were not reported in detail:

- According to OECD 226 for determination of the ECx (e.g. EC10, EC50), twelve concentrations should be tested. At least two replicates for each test concentration and six control replicates are recommended. The spacing factor may vary, i.e. less

	than or equal to 1.8 in the expected effect range and above 1.8 at the higher and lower concentrations. For determination of the NOEC, at least five concentrations in a geometric series should be tested. Four replicates for each test concentration plus eight controls are recommended. The concentrations should be spaced by a factor not exceeding 2.0. A combined approach allows for determination of both the NOEC and EC_x. Eight treatment concentrations in a geometric series should be used. Four replicates for each treatment plus eight controls are recommended. The concentrations should be spaced by a factor not exceeding 1.8.In the current study 5 concentrations with each 4 replicates were tested with a spacing factor of 2. **Acceptability of the analytical methods used in the test:** No information regarding the analytical methods was reported. **Endpoints:** In the study report a NOEC of 1000 mg test item/kg soil dw is suggested. However, at 1000 mg test item/kg soil dw the reproductive effects are 17%. Therefore the endpoint proposed by the RMS is 14 day NOEC = 500 mg ai/kg soil dw 14 day LC_{50} > 1000 mg ai/kg soil dw According to ToxRat® 3.1.0.: $EC_{10\ offspring}$ = 453.7 (95% C.I. = 370.4 – 1534 mg test item/kg soil dw) reliability based on normalised width of C.I. = bad $EC_{20offspring}$ = 988.4 (95% C.I. = 408.4 – 2325.4 mg test item/kg soil dw) $EC_{50offspring}$ = 1660,1 (95% C.I. = 254.7 – 9964.1 mg test item/kg soil dw) **Conclusion of the RMS:** Based on the evaluation of the study the chronic predatory mite toxicity test is considered valid.

Metabolites:

Reference:	**Effects of REG.No.5079285 (metabolite of BAS 595 F, triticonazole) on the reproduction of the collembolan *Folsomia candida***
Author(s), year:	Friedrich, S., 2013d
Report/Doc. number:	BASF DocID 2014/1000028
Guideline(s):	OECD 232 (2009), ISO 11267 (1999)
GLP:	Yes
Deviations:	Please refer to the commenting box below
Validity:	Acceptable

Material and methods:

Test substance:	Reg.No. 5079285 (RPA 404766, metabolite of BAS 595 F, triticonazole); batch no.: L67-148, purity: 99.3% w/w analysed (tolerance ± 1%)
Test species:	Collembola *Folsomia candida* (Willem); in-house culture (originally purchased from "Biologische Bundesanstalt"), Berlin-Dahlem
Number of organisms:	8 replicates per control and solvent control, 4 replicates for the 5 treatment groups, each with 10 individuals. 2 additional replicates per treatment control and solvent control to for measurement purposes.
Life stage, age:	Juveniles 9-12 days old
Type of test, duration:	Chronic laboratory dose-response test, 28 days
Applied concentrations:	Control, solvent control, 31.25, 62.5, 125, 250 and 500 mg test item/kg soil dry weight (based on analysed purity) incorporated into the soil
Solvent:	Acetone
Toxic standard:	Boric acid, Purity: 100% (analysed), the effects of the reference item were investigated in a separate study at concentrations of 44, 67, 100, 150 and 225 mg/kg soil dry weight.
Test substrate:	Artificial soil, 5 % peat, 20 % kaolin clay, 74.7 % industrial quartz sand, 0.3% calcium carbonate;
Substrate/test vessel:	30 g wet weight/test container
Temperature:	18.1– 21.9 °C
Light regime:	16 hours light, 8 hours dark; light intensity: 540 lux
Water content:	Test start: 56.8 – 57.3% of WHC Test end: 55.7-56.8% of WHC
pH:	Test start: 6.03-6.07 Test end: 5.82-5.87
Feeding:	Approximately 2 mg dry yeast at test start and on day 14.
Test parameters:	pH and water content were determined at test start and test end. Water content

maintenance was checked weekly after application.

Mortality of adults, reproductive output (number of juvenile Collembola) was assessed after 28 days.

Statistics: Fisher`s Exact Binomial Test with Bonferroni correction for mortality (α = 0.05, one-sided greater), Williams-t-test for reproduction (α = 0.05, one-sided smaller).

The software used to perform the statistical analysis was ToxRat Professional, Version 2.10.06 (RATTE 2010)

Findings: Differences between the behaviour of the collembolans in the control groups and the test item treatment groups could not be observed.

Table 9.4-7: Effects on mortality and reproduction of *Folsomia candida* in a chronic test

Treatment group [mg ai/kg soil d.w.]	Mean number of surviving parental collembolans after 28 d (± SD)	Mortality of adult Collembola after 28 d [%]	Mean number of juveniles after 28 d (± SD)	Reproduction [% of control]
Control	9.6 (0.7)	3.8	973 (45.3)	-
Solvent control	9.6 (0.7)	3.8	972(65.3)	100
31.25	9.5 (0.6)	5.0	1009 (145.0)	104
62.5	10 (0.0)	0.0	949(46.9)	98
125	9.8 (0.5)	2.5	1005(154.9)	103
250	9.5 (0.6)	5.0	995 (131.7)	102
500	9.5(1.0)	5.0	981 (127.2)	101

SD…Standard Deviation
*statistically significant different compared to control

In the most recent study the LC_{50} and EC_{50} for the reference item boric acid was determined to be 108 mg test item/kg soil dw and 192 mg test item/kg soil dw, respectively. The NOEC for mortality and for reproduction was determined to be 100 and 44 mg test item/kg soil dw, respectively.

Conclusion: NOEC = 500 mg test item/kg soil dw

LC_{50} (mortality) > 500 mg test item/kg soil dw

Comment RMS: The study was evaluated following the recommendations of the currently valid test guideline OECD 232 (2016).

Check of validity criteria:

- Mean adult mortality in the controls should not exceed 20% at the end of the test. In the current study mortality was 3.8% and 3.8% in the control and solvent control, respectively. Fulfilled.
- The mean number of juveniles per vessel in the controls should be at least 100 at the end of the test. In the current study the mean number of juveniles per vessel was 973 and 972 in the control and solvent control, respectively. Fulfilled.

	- The coefficient of variation calculated for the number of juveniles in the controls should be less than 30% at the end of the definitive test. In the current study the coefficient of variation for the pooled control was 5.6%. Fulfilled. **In addition, the following points deviated from the test guideline or were not reported in detail:** - According to OECD 232 for determination of the NOEC/LOEC, at least five concentrations in a geometric series should be tested. Four replicates for each test concentration treatment plus eight controls are recommended. The concentrations should be spaced by a factor not exceeding 1.8. A combined approach allows for determination of both the NOEC/LOEC and EC_x. For this combined approach, eight treatment concentrations in a geometric series should be used. Four replicates for each treatment plus eight controls are recommended. The concentrations should be spaced by a factor not exceeding 1.8. In the current study 5 concentrations with each 4 replicates were tested with a spacing factor of 2. **Acceptability of the analytical methods used in the test:** No Information regarding the analytical methods was reported. **Endpoints:** The RMS agrees on the endpoints given in the study report. 28 day NOEC = 500 mg test item/kg soil dw 28 day LC_{50} > 500 mg test item/kg soil dw According to ToxRat® 3.1.0. EC_{10} values could not be calculated. **Conclusion of the RMS:** Based on the evaluation of the study the chronic collembolan toxicity test is considered valid.

Reference:	**Effect of Reg.No. 5059144 (RPA 406341, metabolite of BAS 595 F) on the reproduction of the Collembola *Folsomia candida* in artificial soil with 5% peat**
Author(s), year:	Royer, S., 2006a
Report/Doc. number:	BASF DocID 2006/1031679
Guideline(s):	ISO 11267: Soil quality-inhibition of reproduction of Collembola (*Folsomia candida*) by soil pollutants, 1999
GLP:	Yes
Deviations:	Please refer to the commenting box below
Validity:	Acceptable

Material and methods:

Test substance:	Reg.No. 5059144 (RPA 406341, metabolite of BAS 595 F); batch no.: BESS0541, purity: 99.0%
Test species:	Collembola *Folsomia candida* (Willem); in-house culture
Number of organisms:	5 replicates per control, solvent control, the 3 treatment groups, each with 10 individuals. 1 additional replicates per treatment control and solvent control to for measurement purposes.
Life stage, age:	Juveniles 10-12 days old
Type of test, duration:	Chronic laboratory dose-response test, 28 days
Applied concentrations:	Control, solvent control, 1, 10, 50, mg/kg soil dry weight incorporated into the soil
Solvent:	Acetone
Toxic standard:	Phenmedipham (BAS 337 01 H); the effects of the reference item were investigated in a separate study.
Test substrate:	Artificial soil, 5 % peat, 20 % kaolin clay, approx. 75 % industrial quartz sand, approx. 0.3% calcium carbonate;
Substrate/test vessel:	30 g wet weight/test container
Temperature:	20 ± 2 °C; not reported in detail
Light regime:	16 hours light, 8 hours dark; light intensity: 450-780 lux
Water content:	Test start: 16.79 – 17.20 % (equivalent to 59.6-61.0 % of WHC) Test end: 16.50-17.01 % (equivalent to 58.6-60.4 % of WHC)
pH:	Test start: 5.55-5.65 Test end: 5.34-5.66
Feeding:	Approximately 2 mg granulated yeast at test start and on day 14.
Test parameters:	pH and water content were determined at test start and test end. Water content was checked at the beginning, after 14 days and at the end of the test. Mortality of adults, reproductive output (number of juvenile Collembola) was assessed after 28 days.

Statistics: Shapiro-Wilk's Test for Normality and Bartlett's Test for Homogeneity of Variance were made. NOEC/LOEC was evaluated by Analysis of Variance and Dunnett Test. The software used was TOXSTAT 3.5.

Findings: No behavioural changes or abnormalities of the Collembola were observed.

Table 9.4-8: Effects on mortality and reproduction of *Folsomia candida* in a chronic test

Treatment group [mg ai/kg soil d.w.]	Mean number of surviving parental collembolans after 28 d	Mortality of adult Collembola after 28 d [%]	Mean number of juveniles after 28 d (± SD)	Reproduction [% of solvent control]
Control	0.2	2.0	434.3 (68.8)	-
Solvent control	0.0	0.0	356.1 (64.1)	-
1	0.6	6.0 n.s.	332.8 (60.0) n.s.	93.5 n.s.
10	0.0	0.0 n.s.	369.1 (95.1) n.s.	103.7 n.s.
50	0.4	4.0 n.s.	350.9 (51.8) n.s.	98.5 n.s.

SD…Standard Deviation
n.s.…not statistically significant different compared to solvent control

In the most recent study the LC_{50} for the reference item Phenmedipham was determined to be 23.2mg test item/kg soil dw. The NOEC for mortality and for reproduction was determined to be 7.9 mg test item/kg soil dw,

Conclusion: NOEC = 50 mg test item/kg soil dw
LC_{50} > 50 mg test item/kg soil dw

Comment RMS: The study was evaluated following the recommendations of the currently valid test guideline OECD 232 (2016).

Check of validity criteria:

- Mean adult mortality in the controls should not exceed 20% at the end of the test. In the current study mortality was 2.0% and 0.0% in the control and solvent control, respectively. Fulfilled.
- The mean number of juveniles per vessel in the controls should be at least 100 at the end of the test. In the current study the mean number of juveniles per vessel was 434.3 and 356.1 in the control and solvent control, respectively. Fulfilled.
- The coefficient of variation calculated for the number of juveniles in the controls should be less than 30% at the end of the definitive test. In the current study the coefficient of variation for the pooled controls was 19%. Fulfilled.

In addition, the following points deviated from the test guideline or were not reported in detail:

- According to OECD 232 for determination of the NOEC/LOEC, at least five concentrations in a geometric series should be tested. Four replicates for each test

	concentration treatment plus eight controls are recommended. The concentrations should be spaced by a factor not exceeding 1.8. A combined approach allows for determination of both the NOEC/LOEC and EC_x. For this combined approach, eight treatment concentrations in a geometric series should be used. Four replicates for each treatment plus eight controls are recommended. The concentrations should be spaced by a factor not exceeding 1.8. In the current study 3 concentrations with each 5 replicates were tested with a spacing factor of 10 and 5 respectively. **Acceptability of the analytical methods used in the test:** No information regarding the analytical methods was reported. **Endpoints:** The RMS agrees on the endpoints given in the study report. Although the number of juveniles in the control and in the solvent control do quite differ no statistically significant difference could be determined. Therefore the two controls were pooled. 28 day NOEC = 50 mg test item/kg soil dw 28 day LC_{50} > 50 mg test item/kg soil dw According to ToxRat® 3.1.0. EC_{10} values could not be calculated. **Conclusion of the RMS:** Based on the evaluation of the study the chronic collembolan toxicity test is considered valid.

Reference:	**Effect of Reg.No. 5079288 (metabolite of BAS 595 F, triticonazole) on the reproduction of the Collembola *Folsomia candida***
Author(s), year:	Friedrich, S., 2013e
Report/Doc. number:	BASF DocID 2014/1000029
Guideline(s):	OECD 232 (2009), ISO 11267 (1999)
GLP:	Yes
Deviations:	Please refer to the commenting box below
Validity:	Acceptable

Material and methods:

Test substance:	Reg.No. 5079288 (RPA 407922, metabolite of BAS 595 F, triticonazole); batch no.: 33484-39, purity: 99.5%, analysed (± 1.0% tolerance)
Test species:	Collembola *Folsomia candida* (Willem); in-house culture (originally purchased from "Biologische Bundesanstalt", Berlin-Dahlheim)
Number of organisms:	8 replicates per control and solvent control, 4 replicates for the 5 treatment groups, each with 10 individuals. 2 additional replicates per treatment control and solvent control to for

	measurement purposes.
Life stage, age:	Juveniles 9-12 days old
Type of test, duration:	Chronic laboratory dose-response test, 28 days
Applied concentrations:	Control, solvent control, 31.25, 62.5, 125, 250 and 500 mg test item/kg soil dry weight (based on analysed purity) incorporated into the soil
Solvent:	Acetone
Toxic standard:	Boric acid, purity: 100% (analysed), the effects of the reference item were investigated in a separate study at concentrations of 44, 67, 100, 150 and 225 mg/kg soil dry weight.
Test substrate:	Artificial soil, 5 % peat, 20 % kaolin clay, 74.7 % industrial quartz sand, 0.3% calcium carbonate;
Substrate/test vessel:	30 g wet weight/test container
Temperature:	18.1– 21.9 °C
Light regime:	16 hours light, 8 hours dark; light intensity: 540 lux
Water content:	Test start: 57.1% of WHC Test end: 55.9-56.6% of WHC
pH:	Test start: 5.88-6.20 Test end: 5.82-5.96
Feeding:	Approximately 2 mg granulated dry yeast at test start and on day 14.
Test parameters:	pH and water content were determined at test start and test end. Water content maintenance was checked weekly after application. Mortality of adults, reproductive output (number of juvenile Collembola) was assessed after 28 days.
Statistics:	Fisher`s Exact Binomial Test with Bonferroni correction for mortality ($\alpha = 0.05$, one-sided greater), Williams-t-test for reproduction ($\alpha = 0.05$, one-sided smaller). The software used to perform the statistical analysis was ToxRat Professional, Version 2.10.06 (RATTE 2010)
Findings:	Differences between the behaviour of the collembolans in the control groups and the test item treatment groups could not be observed.

Table 9.4-9: Effects on mortality and reproduction of *Folsomia candida* in a chronic test

Treatment group [mg ai/kg soil d.w.]	Mean number of surviving parental collembolans after 28 d (± SD)	Mortality of adult Collembola after 28 d [%]	Mean number of juveniles after 28 d (± SD)	Reproduction [% of solvent control]
Control	9.8 (0.5)	2.5	704 (68.5)	-
Solvent control	9.8 (0.5)	2.5	720 (77.4)	100
31.25	9.8 (0.5)	2.5	724 (62.0)	101
62.5	9.8 (0.5)	2.5	721 (121.5)	100
125	9.8 (0.5)	2.5	742 (164.5)	103

Treatment group [mg ai/kg soil d.w.]	Mean number of surviving parental collembolans after 28 d (± SD)	Mortality of adult Collembola after 28 d [%]	Mean number of juveniles after 28 d (± SD)	Reproduction [% of solvent control]
250	9.8 (0.5)	2.5	718 (111.3)	100
500	9.0 (0.8)	10.0	611 (57.7)	85

SD…Standard Deviation

In the most recent study the LC_{50} and EC_{50} for the reference item boric acid was determined to be 108 mg test item/kg soil dw and 192 mg test item/kg soil dw, respectively. The NOEC for mortality and for reproduction was determined to be 100 and 44 mg test item/kg soil dw, respectively.

Conclusion: NOEC = 500 mg test item/kg soil dw

LC_{50} > 500 mg test item/kg soil dw

Comment RMS: The study was evaluated following the recommendations of the currently valid test guideline OECD 232 (2016).

Check of validity criteria:

- Mean adult mortality in the controls should not exceed 20% at the end of the test. In the current study mortality was 2.5% and 2.5% in the control and solvent control, respectively. Fulfilled.
- The mean number of juveniles per vessel in the controls should be at least 100 at the end of the test. In the current study the mean number of juveniles per vessel was 704 and 720 in the control and solvent control, respectively. Fulfilled.
- The coefficient of variation calculated for the number of juveniles in the controls should be less than 30% at the end of the definitive test. In the current study the coefficient of variation was 10% for the pooled controls. Fulfilled.

In addition, the following points deviated from the test guideline or were not reported in detail:

- According to OECD 232 for determination of the NOEC/LOEC, at least five concentrations in a geometric series should be tested. Four replicates for each test concentration treatment plus eight controls are recommended. The concentrations should be spaced by a factor not exceeding 1.8.

A combined approach allows for determination of both the NOEC/LOEC and EC_x. For this combined approach, eight treatment concentrations in a geometric series should be used. Four replicates for each treatment plus eight controls are recommended. The concentrations should be spaced by a factor not exceeding 1.8. In the current study 5 concentrations with each 4 replicates were tested with a spacing factor of 2.

Acceptability of the analytical methods used in the test: No information

	regarding the analytical methods was reported. **Endpoints:** In the study report a NOEC of 500 mg test item/kg soil dw is suggested. However, at 500 mg test item/kg soil dw the reproductive effects are 15%. Therefore the endpoint proposed by the RMS is 28 day NOEC = 250 mg test item/kg soil dw 28 day LC_{50} > 500 mg test item/kg soil dw According to ToxRat® 3.1.0. $EC_{10\ offspring}$ = 457.442 (95% C.I. = 130.831-1599.421 mg test item/kg soil dw) reliability based on normalised width of C.I. = bad **Conclusion of the RMS:** Based on the evaluation of the study the chronic collembolan toxicity test is considered valid.

Reference:	**Effects of Reg.No. 5059144 (metabolite of BAS 595 F) on reproduction of the predatory mite *Hypoaspis aculeifer* in artificial soil with 5% peat**
Author(s), year:	Ganßmann, M., 2014a
Report/Doc. number:	BASF DocID 2014/1083348
Guideline(s):	OECD 226 (2008)
GLP:	Yes
Deviations:	Please refer to the commenting box below
Validity:	Acceptable

Material and methods:

Test substance:	Reg. No. 5059144 (metabolite of BAS 595 F, RPA 406341), batch no. L74-160, purity: 91.8%
Test species:	Predatory mites, *Hypoaspis aculeifer* (CANESTRINI); cultured by IBACON
Number of organisms:	8 replicates for the control and 4 replicates per treatment group, 1 additional replicate per treatment and control to check the pH and water content of the test substrate after 14 days, each with 10 individuals.
Life stage:	Adult females, approx. 14 days after reaching the adult stage
Type of test, duration:	Laboratory sub-lethal test, 14 days
Applied concentrations:	Control, 0.625, 1.25, 2.5, 5.0 and 10.0 mg test item/kg soil dw incorporated into the soil
Solvent:	Acetone
Toxic standard:	Perfekthion, content of dimethoate nominal: 400.0 g/L, analysed: 411.7 g/L; the reference item test (dose response) is performed at least once a year at the test facility.
Test substrate:	Artificial soil, 5 % sphagnum peat, 20 % kaolin clay, 74.8 % industrial quartz

	sand, 0.2% calcium carbonate
Substrate/test vessel:	20 g dry weight/test container
Temperature:	18 – 22 °C
Light regime:	16 hours light, 8 hours dark; light intensity: 400 to 800 lux
Water content:	Test start: 23.7 – 24.2% (equivalent to 56.4 – 57.5% of WHC)
	Test end: 23.1 – 23.9% (equivalent of 55.0 – 57.0% of WHC)
pH:	Test start: 6.1 – 6.2
	Test end: 6.3
Feeding:	The predatory mites were fed with cheese mites (*Tyrophagus putrescentiae*), at the experimental start and on day 2, 4, 6, 9 and 11.
Test parameters:	pH and water content were determined at test start and test end. Water content maintenance was checked on day 7 after application.
	Mortality of adults, differences in morphology, behavioural effects and number of juveniles were assessed after 14 days
Statistics:	Mortality: Fisher's Exact Test (multiple comparison, $\alpha = 0.05$, one-sided greater). An LC_{50} value and its 95% confidence limits were calculated by applying Probit-Analysis (Finney, 1971). Values were compensated for control mortality using Abbott's formula.
	Reproduction: Williams t-test (multiple comparison, $\alpha = 0.05$, one-sided smaller).
	The software used to perform the statistical analysis was ToxRat Professional, Version 2.10.05, ® ToxRat Solutions GmbH.
Findings:	
Biological effects:	No differences in behaviour and morphology of the mites between the test item groups and the control were observed.

Table 9.4-10: Effects on mortality and reproduction of *Hypoaspis aculeifer* in a sub-chronic test

Treatment group [mg ai/kg soil d.w.]	Mean number of surviving adult mites after 14 d	Mortality of adult mites after 14 d [%] (± SD)	Statistical evaluation	Mean number of juvenile mites after 14 d (± SD)	Reproduction [% of solvent control]	Statistical evaluation
Control	8.8	13 (10)	-	161 (34)	100	-
0.625	9.3	8 (15)	n.s.	189 (11)	117	n.s.
1.25	9.5	5 (6)	n.s.	189 (27)	117	n.s.
2.5	8.8	13 (5)	n.s.	198 (20)	123	n.s.
5	9.5	5 (6)	n.s.	186 (21)	116	n.s.
10	9.25	8 (5)	n.s.	175 (11)	109	n.s.

SD…Standard Deviation
n.s….not statistically significant different compared to control

Dimethoate showed an EC_{50} of 4.2 mg /kg soil dw. The NOEC based on reproduction and mortality was calculated to be 2.0 mg /kg soil dw. This shows that the test organisms are sufficiently sensitive.

Conclusion:	14 day LC_{50} / EC_{50} (mortality, reproduction) > 10 mg test item/kg soil dw 14 day NOEC (mortality, reproduction) = 10 mg test item/kg soil dw
Comment RMS:	The study was evaluated following the recommendations of the currently valid test guideline OECD 226 (2016). **Check of validity criteria:** - Mean adult mortality of the females in the controls should not exceed 20% at the end of the test. In the current study mortality was 13 % in the control Fulfilled. - The mean number of juveniles per replicate in the controls should be at least 50 at the end of the test. In the current study the mean number of juveniles per vessel was 161 in the control. Fulfilled. - The coefficient of variation calculated for the number of juvenile mites in the controls should not be higher than 30% at the end of the definitive test. In the current study the coefficient of variation was 21.3% for the control. Fulfilled. **In addition, the following points deviated from the test guideline or were not reported in detail:** - According to OECD 226 for determination of the EC_x (e.g. EC_{10}, EC_{50}), twelve concentrations should be tested. At least two replicates for each test concentration and six control replicates are recommended. The spacing factor may vary, i.e. less than or equal to 1.8 in the expected effect range and above 1.8 at the higher and lower concentrations. For determination of the NOEC, at least five concentrations in a geometric series should be tested. Four replicates for each test concentration plus eight controls are recommended. The concentrations should be spaced by a factor not exceeding 2.0. A combined approach allows for determination of both the NOEC and EC_x. Eight treatment concentrations in a geometric series should be used. Four replicates for each treatment plus eight controls are recommended. The concentrations should be spaced by a factor not exceeding 1.8.In the current study 5 concentrations with each 4 replicates were tested with a spacing factor of 2. **Acceptability of the analytical methods used in the test:** No information regarding the analytical methods was reported. **Endpoints:** The RMS agrees on the endpoints given in the study report. 14 day NOEC = 10 mg test item/kg soil dw 14 day LC_{50} > 10 mg test item/kg soil dw According to ToxRat® 3.1.0. EC_x values could not be calculated. **Conclusion of the RMS:** All treatment groups showed a higher number of juveniles than in the control group (9 to 23%). However, the Guideline recommends a one-tailed (smaller) hypothesis testing, not considering an increase

	in reproduction as an adverse effect. Based on the evaluation of the study the chronic predatory mite toxicity test is considered valid.

B.9.5. EFFECTS ON SOIL NITROGEN TRANSFORMATION

In the first EU peer-review of the active substance triticonazole a study on nitrogen and carbon mineralisation was submitted addressing the risk to soil micro-flora (Aldred & Seal, 1991). According to the EU data requirements for active substances (Regulation No. 283/2013) and plant protection products (Regulation No. 284/2013) the impact on soil microbial activity should be evaluated, in terms of nitrogen transformation. Hence, the available study was only re-evaluated considering the nitrogen transformation. Information regarding carbon mineralisation was not re-evaluated. For the current submission, nitrogen transformation tests have been provided with the formulation BAS 595 01 F (please refer to Volume 3 B.9-CP) as well as for the metabolites RPA 406341, RPA 404766 and RPA 407922. Summaries and evaluations of the studies are presented in the following. For the metabolite RPA 406341, also a carbon transformation test has been provided. For this study only the reference is presented, but it has not been evaluated by the RMS as carbon transformation testing is no longer a data requirement.

Active substance:

Reference:	**A laboratory assessment of the effects of RPA 400727 on soil microflora respiration and nitrogen turnover and report amendment**
Author(s), year:	Aldred, D., Seal, K.J., 1991 and Leake, C.R., 1998
Report/Doc. number:	R013039 and C017774
Guideline(s):	BBA 1989 part VI, 1-1
GLP:	Yes
Deviations:	Please refer to the commenting box below
Validity:	Not possible to verify

Material and methods:

Test substance:	RPA 400727; batch no.: DA 646; purity: 97.1%
Test species:	Soil microflora
Type of test, duration:	Nitrogen transformation test, 28 days
Applied concentrations:	0 (control), 0.32 and 1.6 kg ai/ha, 3 replicates per treatment group
Source of organic nitrogen supplement:	Alfalfa, ground to pass a 0.5 mm sieve, containing 36.7% organic carbon and 2.3% total nitrogen
Solvent/vehicle:	None
Toxic standard:	Dinoseb acetate; no further information reported
Test substrate:	Sandy-clay loam and clay loam

	Sandy-clay loam: organic carbon: 0.85 %, pH: 6.7, Clay: 13%, Silt: 13%, Sand: 74%; maximum water holding capacity: 36.43%; achieved moisture: 12.50 % Clay loam: organic carbon: 1.55 %, pH: 6.1, Clay: 22%, Silt: 20%, Sand: 58%; maximum water holding capacity: 36.43%, achieved moisture: 18.80%
Substrate/test vessel:	1-1.5 kg soil dw per replicate
Incubation:	22 ± 1°C
Water content	Test start: 40% ± 5 of WHC Test end: not specified
pH:	Test start: 6.7 and 6.1, respectively Test end: not reported
Test parameters:	Nitrogen transformation was followed by measuring the amounts of NH^+_4-N, NO^-_2-N and NO^-_2-N in soil. The nitrogen transformation was determined on day 0 and at intervals of 7, 14, 28 after application. The samples were extracted with 100 ml deionised water by shaking for sixty minutes, settling and centrifuging the supernatant at 3600 rpm for five minutes. The clear liquid was decanted off and refrigerated at 4°C. Analysis for ammonia, nitrate and nitrite was either done by steam distillation or colorimetric methods within 24 hours. Which method has been used is not reported in detail.
Conclusion:	No significant differences were found between the treatments in either of the soils after 28 days with the exception of the ammonium-nitrogen level in the sandy-clay loam at 1.8 kg ai/ha.

Table 9.5-1: Effects of triticonazole on nitrogen transformation in soil

Treatment group [g ai/ha]	Sandy-clay loam soil						Clay loam soil					
	Ammonium-nitrogen level [mg nitrogen/kg soil]			Nitrate-nitrogen levels in alfalfa amended soil [mg nitrogen/kg soil]			Ammonium-nitrogen level [mg nitrogen/kg soil]			Nitrate-nitrogen levels in alfalfa amended soil [mg nitrogen/kg soil]		
	Days after application											
	0	14	28	0	14	28	0	14	28	0	14	28
Control	9.9	5.1	2.2	16.0	54.0	67.2	14.9	5.4	4.7	24.9	60.9	69.3
320	9.9	5.7	4.1	16.0	49.5	64.5	14.9	4.6	3.9	24.9	46.4	69.1
1600	9.9	8.1	6.9*	16.0	47.2	58.8	14.9	4.1	5.0	24.9	58.3	64.8

*significantly different to the control

Comment RMS:	The study was evaluated following the recommendations of the currently valid test guideline OECD 216 (2000). According to the study report amendment, the application rate should be reported as 320 g ai/ha and 1600 g ai/ha instead of 360 g ai/ha and 1800 g ai/ha. This was already amended in the summary above.

Check of validity criteria:

- Evaluations of test results with agrochemicals are based on relatively small differences (i.e. average value ± 25 %) between nitrate concentrations in control and treated soil samples, so large variations in the controls can lead to false results. Therefore, the variation between replicate control samples should be less than ± 15%. In the current study the variation between the replicate control samples was not reported. No raw data is given and therefore a calculation of the variation was not possible.

In addition, the following points deviated from the test guideline or were not reported in detail:

- According to OECD 216 one single soil is used for the test. In the current studies two soils were tested.
- According to OECD 216 the organic carbon content of the soil should be: 0.5 - 1.5 %. In the current study the organic carbon content for the clay loam soil was 1.55 %.
- According to OECD 216 the microbial biomass should be measured and its carbon content should be at least1 % of the total soil organic carbon. In the current study the % of biomass carbon content was not reported.
- According to OECD 216 detailed information on the history of the field site from where the test soil is collected should be available. In the current study no such information is given.
- According to OECD 216 the moisture content of soil samples should be maintained during the test between 40 % and 60 % of the maximum water holding capacity of the soil with a range of ± 5 %. In the current study it is not reported if the moisture content was maintained until the end of the test.
- According to OECD 216 the quantity of nitrate formed in each replicate soil sample should be recorded, and the mean values of all replicates should be provided in tabular form. Nitrogen transformation rates should be evaluated by appropriate and generally acceptable statistical methods (e.g. F-test, 5% significance level). The quantities of nitrate formed are expressed in mg nitrate/kg dry weight soil/day. The nitrate formation rate in each treatment is compared with that in the control, and the percent deviation from the control is calculated. In the current study no values for the single replicates are given and the nitrogen levels were expressed in mg nitrogen/kg soil.

Acceptability of the analytical methods used in the test: No clear information given. A document for the operationg procedure for the ADC gas handling unit and the infra-red CO_2 analyser was provided in Appendix 1.

Endpoints:

At 320 g ai/ha no significant effects could be observed. At 1600 g ai/ha, significant

	effects on ammonium nitrogen level was observed after 28 days. **Conclusion of the RMS:** It was not possible to evaluate the reliability of this study due to the lack of reported data for analytical methods, variation between replicates, quantities of nitrate formed and other parameters related to the soil test samples. Based on the evaluation of the study it is not considered reliable.

Metabolites:

Reference:	**Effect of Reg.No. 5059144 (RPA 406341, metabolite of BAS 595 F) on soil microorganisms: carbon transformation test**
Author(s), year:	Royer, S., 2006c
Report/Doc. number:	BASF DocID 2006/1028714
Guideline(s):	OECD 216 (2000)
GLP:	Yes

Reference:	**Effect of Reg.No. 5059144 (RPA 406341, metabolite of BAS 595 F) on soil microorganisms: nitrogen transformation test**
Author(s), year:	Royer, S., 2006b
Report/Doc. number:	BASF DocID 2006/1028713
Guideline(s):	OECD 216 (2000)
GLP:	Yes
Deviations:	Please refer to the commenting box below
Validity:	Acceptable

Material and methods:

Test substance:	Reg.No. 5059144, batch no.: BESS0541, purity: 99.0%
Test species:	Soil microflora
Type of test, duration:	Nitrogen transformation test, 28 days
Applied concentrations:	Control, 1 and 10 mg test item/kg dry soil, 3 replicates per control and treatment group
Solvent/vehicle:	Acetone
Toxic standard:	Dinoterb, tested at concentrations of 6.7 and 13.3 mg/kg soil dw, 3 replicates per treatment group
Test substrate:	Silty sand soil (loamy sand), removed to a depth of 20 cm, from a field located in Offenbach, Germany. No application of fertilisers and plant protection products applied in the sampling year and 4 former years. Total organic Carbon 1.12 %, pH ($CaCl_2$): 6.7, pH (H_2O): 7.5, Carbon content of

	microbial biomass: 19 gC/100 g soil dw (corresponding to 1.7% of organic C) Total nitrogen content: 0.09% Water holding capacity (WHC): 34.2 g/100 g soil dw Texture according to DIN 11277: 6.7 % clay, 34.4 % silt, 58.9 % sand 0.5% (i.e. 7.5 g/ 1656 g fresh soil) lucerne meal
Substrate/test vessel:	50 g soil dw/500 mL glass flasks
Incubation:	20°C ±2, darkness
Water content	Approx. 55% of WHC
pH:	Test start: 6.65 – 6.71 Test end: 6.95 – 7.04
Test parameters:	The nitrogen transformation was determined0, 7, 14 and 28 days after application. The NH_4-N formed from organically bound nitrogen in the Lucerne meal and the NO_3-N from the nitrification process was determined by using an ammonia-electrode and a nitrate- and reference-electrode, respectively. The soil samples were mixed with an aqueous solution of 1% AIK(So4)2 to reach at total amount of 200 mL. The suspensions were shaken for one hour and filtered through a folded filter. NO_3-N concentrations were measured immediately afterwards. The NH_4-N concentrations were determined after addition of 1 mL of 10 M NaOH.
Statistics:	The mean values per sampling date were converted to mg NO_3-N/kg dry soil and to mg NO_3-N/kg dry soil/day. Calculation of mean values per treatment, standard deviations and coefficients of variation.

Findings:

Table 9.5-2: Effects of Reg.No. 5059144 (RPA 406341) on nitrogen transformation

Sampling date (DAA)	Treatment	Measured values [mg NO_3-N/ 50g sdw]	Mean value [mg NO_3-N/ 50g sdw] (SD)	CV [%]	mg NO_3-N /kg sdw	mg NO_3-N/ kg sdw/day	Deviation from control [%]
0	Control	0.47	0.47 (0.01)	2.1	9.4	-	-
		0.47					
		0.46					
	1 mg/kg sdw	0.47	0.47 (0.0)	0.0	9.4	-	0.00
		0.47					
		0.47					
	10 mg/kg sdw	0.47	0.48 (0.01)	2.1	9.6	-	2.13
		0.48					
		0.48					
7*	Control	0.10	0.10 (0.01)	10.0	2.0	0.29	-
		0.09					

		0.10					
	1 mg/kg sdw	0.09 0.09 0.09	0.09 (0.00)	0.0	1.8	0.26	-10.00
	10 mg/kg sdw	0.09 0.08 0.09	0.09 (0.01)	11.1	1.8	0.26	-10.00
14	Control	0.19 0.21 0.22	0.21 (0.02)	9.5	4.2	0.30	-
	1 mg/kg sdw	0.21 0.21 0.20	0.21 (0.01)	4.8	4.2	0.30	0.00
	10 mg/kg sdw	0.16 0.18 0.19	0.18 (0.02)	11.1	3.6	0.26	-14.29
28	Control	0.74 0.73 0.73	0.73 (0.01)	1.4	14.6	0.52	-
	1 mg/kg sdw	0.66 0.66 0.65	0.66 (0.01)	1.5	13.2	0.47	-9.59
	10 mg/kg sdw	0.64 0.64 0.65	0.64 (0.01)	1.6	12.8	0.46	-12.33

sdw…soil dry weight
DAA…Days after application
CV %…Coefficient of Variation in %
*All NO_3-N-values of these days are below Limit of Quantification. Nevertheless the values were involved in the evaluations for the corresponding days. So the development of the deviations from control after different days can be demonstrated. Relevant for the conclusion of the study is only the deviation from control after 28 days.
MV…mean value, SD…standard deviation – Limit of Quantification: 0.5 mg/50 g dry soil (DAA 0, 7, 28) or 0.1 mg/50 g dry soil (DAA 14)

The reference item Dinoterb caused a stimulation of nitrogen transformation of + 28.77 % and + 39.73 % at 6.7 mg and 13.3 mg Dinoterb per kg soil dry weight, respectively, determined 28 days after application.

Conclusion: Reg.No. 5059144 (RPA 406341) caused no adverse effects on the soil N-transformation (measured as NO_3-N production) at the end of the 28-day incubation period.

Comment RMS:	The study was evaluated following the recommendations of the currently valid test guideline OECD 216 (2000). **Check of validity criteria:**

	- Evaluations of test results with agrochemicals are based on relatively small differences (i.e. average value ± 25 %) between nitrate concentrations in control and treated soil samples, so large variations in the controls can lead to false results. Therefore, the variation between replicate control samples should be less than ± 15%. In the current study the variation coefficient of replicate control samples ranged between 1.4 and 10% for the single sampling dates. The overall coefficient of variation for the control, estimated by ToxRat® 3.1.0., was 0.8%. Fulfilled. **Acceptability of the analytical methods used in the test:** Evaluation of the analytical methods (water/sediment/soil) according to the Guidance Document SANCO/3029/99 rev. 4. Method: EA 940 and Nitrate-electrode 93-07 Calibration solutions: 0.1, 0.5 and 10 mg NO_3-N/200 ml; 0.5 and 10 mg NH_4-N/200 ml Befor starting the measurements the system was calibrated with the calibration solutions. LOQ: 10 mg/kg soil dw LOD: not reported **Endpoints:** Only the mean transformation rate over the replicates of one sampling was provided in the study report, transformation rates per replicate were not reported and could therefore not be recalculated by the RMS with ToxRat® 3.1.0. However, the data presented in the study report appears to be plausible and is considered acceptable. 28 day EC_{25} > 10 mg test item/kg soil dw **Conclusion of the RMS:** Based on the evaluation of the study the nitrogen transformation test on soil microorganisms is considered valid.

Reference:	**Reg.No. 5079285 (metabolite of BAS 595F, triticonazole): Effects on the activity of soil microflora under laboratory conditions (nitrogen transformation)**
Author(s), year:	Stojanowitsch, née Gehrig, M., 2015a
Report/Doc. number:	BASF DocID 2013/1103636
Guideline(s):	OECD 216 (2000)
GLP:	Yes
Deviations:	Please refer to the commenting box below
Validity:	Acceptable

Material and methods:

Test substance:	Reg.No. 5079285 (RPA 404766, metabolite of BAS 595F, triticonazole,), batch

	no.: L67-148, analysed purity: 99.3% (± 1.0%)
Test species:	Soil microflora
Type of test, duration:	Nitrogen transformation test, 42 days
Applied concentrations:	Control, 0.1 and 1.0 mg test item/kg soil dw, 3 replicates
Solvent/vehicle:	None
Toxic standard:	Sodium Chloride, tested at concentrations of 20 g/kg soil dw
Test conditions:	
Test substrate:	Silty sand soil (loamy sand), removed to a depth of 20 cm, from a field located in Offenbach, Germany. No application plant protection products for at least four years prior to soil sampling. No application of organic fertilisers at least six month and no mineral fertilizer for four years prior to soil sampling. Total organic Carbon 1.05 %, pH ($CaCl_2$): 7.0, Carbon content of microbial biomass: 74.2 mg C/100 g soil dw (corresponding to 7.07% of TOC) Total nitrogen content: 0.12% Water holding capacity (WHC): 39.86% Texture according to DIN: 9.7 % clay, 36.2 % silt, 54.1 % sand 0.5% (i.e. 7.5 g/ 3300 g pre-moistened soil) lucerne meal
Substrate/test vessel:	2910.6 g soil dw
Incubation:	19.4 – 21.1°C, darkness
Water content	13.2-14.4% (soil wet weight)
pH:	7.0-7.4
Test parameters:	The nitrogen transformation was determined on day 0, and at intervals of 7, 14, 28 and 42 days after application. 50 g soil dw were made up to a final volume of 200 mL with a 0.1% alum solution. The samples were vigorously shaken for approx. 30 minutes. The aqueous supernatant was filtered and used for the nitrate measurements using a NO_3^- selective electrode.
Statistics:	The mean nitrogen-content, standard deviation and coefficient of variation were calculated for each treatment group and sampling date. Furthermore, the evaluation was based on mean nitrate concentrations at the different sampling dates and on the mean nitrate formation rates (mg/kg per day) at time intervals between each sampling day and day 0.

Findings:

Table 9.5-3: Effects of Reg.No. 5079285 (RPA 404766) on nitrogen transformation

Sampling date (DAA)	Treatment	Measured values [mg NO_3-N/ kg sdw]	Mean value [mg NO_3-N/ kg sdw]	Deviation from control [%]	CV [%]	mg NO_3-N/ kg sdw/day	Deviation from control [%]
0	Control	25.1 23.7	24.2	-	3.42	-	-

		23.8					
	0.1 mg/kg sdw	28.5	27.1	+12.1	6.17	-	-
		27.6					
		25.3					
	1.0 mg/kg sdw	26.3	27.2	+12.5	4.06	-	-
		26.9					
		28.4					
7	Control	8.56	7.53	-	12.5	-2.38	-
		6.72					
		7.31					
	0.1 mg/kg sdw	11.3	8.77	+16.5	26.9	-2.62	-10.1
		6.61					
		8.42					
	1.0 mg/kg sdw	13.8	12.3	+63.9	11.3	-2.12	+10.7
		12.2					
		11.0					
14	Control	7.13	6.78	-	7.77	-1.24	-
		6.18					
		7.05					
	0.1 mg/kg sdw	7.93	6.92	+2.07	15.0	-1.44	-16.0
		6.99					
		5.85					
	1.0 mg/kg sdw	7.34	8.52	+25.5	12.0	-1.33	-7.41
		9.03					
		9.18					
28	Control	16.0	14.7	-	8.37	-0.337	-
		13.5					
		14.8					
	0.1 mg/kg sdw	16.2	15.1	+2.72	6.51	-0.0428	-26.8
		15.0					
		14.2					
	1.0 mg/kg sdw	13.0	15.2	+3.25	13.0	-0.428	-26.9
		15.9					
		16.8					
42	Control	50.3	47.7	-	5.16	0.560	-
		45.5					
		47.3					
	0.1 mg/kg sdw	47.7	49.5	3.81	6.71	0.534	-4.73
		53.4					
		47.5					
	1.0 mg/kg sdw	39.9	44.9	-5.89	9.71	0.421	-24.8
		47.8					

		47.0					

sdw…soil dry weight
DAA…Days after application
CV %...Coefficient of Variation in %
+…increase of nitrogen transformation; -…decrease of nitrogen transformation

In a separate study the reference item Sodium Chloride caused an effect on nitrogen transformation of 88.5 %, 28 days after application.

Conclusion: Reg.No. 5079285 caused no long-term adverse effects (difference to control < 25 %, OECD 216) on the soil nitrogen transformation (expressed as NO_3-N production) at the end of 42-day incubation period.

Comment RMS: The study was evaluated following the recommendations of the currently valid test guideline OECD 216 (2000).

Check of validity criteria:

- Evaluations of test results with agrochemicals are based on relatively small differences (i.e. average value ± 25 %) between nitrate concentrations in control and treated soil samples, so large variations in the controls can lead to false results. Therefore, the variation between replicate control samples should be less than ± 15%. In the current study the variation coefficient of replicate control samples ranged between 3.42 and 12.5% for the single sampling dates. The overall coefficient of variation for the control, estimated by ToxRat® 3.1.0., was 5.1%. Fulfilled.

Acceptability of the analytical methods used in the test:

Method: pH/ION 735; control used for calibration

Linearity: calibration range 5.0 - 100 mg NO_3/L

Precision: Coefficients of variation < 15%

Endpoints: Only the mean transformation rate over the replicates of one sampling was provided in the study report, transformation rates per replicate were not reported and could therefore not be recalculated by the RMS with ToxRat® 3.1.0. However, the data presented in the study report appears to be plausible and is considered acceptable.

42 day EC_{25} > 1 mg test item/kg soil dw

Conclusion of the RMS: Based on the evaluation of the study the nitrogen transformation test on soil microorganisms is considered valid.

Reference: **Effects of Reg.No. 5079288 (metabolite of BAS 595F, triticonazole) on the activity of soil microflora (nitrogen transformation test)**

Author(s), year: Schulz, L., 2014b

Report/Doc. number:	BASF DocID 2014/1000025
Guideline(s):	OECD 216 (2000)
GLP:	Yes
Deviations:	Please refer to the commenting box below
Validity:	Acceptable

Material and methods:

Test substance:	Reg.No. 5079288 (RPA 407922, metabolite of BAS 595F, triticonazole), batch no.: 33484-39, analysed purity: 99.5% (tolerance ± 1.0%)
Test species:	Soil microflora
Type of test, duration:	Nitrogen transformation test, 28 days
Applied concentrations:	Control, 0.1 and 1.0 mg test item/kg soil dw , 3 replicates
Solvent/vehicle:	None
Toxic standard:	Dinoterb, tested in a separate study at concentrations of 6.8, 16.00 and 27.00 mg/kg soil dw

Test conditions:

Test substrate:	Agriculturally utilised soil (loamy sand), removed to a depth of 20 cm, from a field located in Canitz, Germany. No application of fertilizers and plant protection products since 2003. organic Carbon 1.47 %, pH: 6.5, Humus content: 2.53%, Carbon content of microbial biomass: 30.56 mgC/100 g soil dw (corresponding to 2.08% of organic C) Total nitrogen content: 0.14% Water holding capacity (WHC): 36.71%, water content [g/100 g soil d.w.]: 9.59 Texture according to ISO 11277: 8.2 % clay, 34.7 % silt, 57.1 % sand 0.5% (i.e. 1 g/ 200 g soil dw) lucerne meal
Substrate/test vessel:	200 g soil dw
Incubation:	19.0 – 20.8°C, darkness
Water content	16.75-17.87 g/100 g soil dw (equivalent 45.62-48.67 % of WHC)
pH:	Test start: 6.2-6.3 Test end: 6.3-6.4
Test parameters:	The nitrogen transformation was determined on day 0 (after approx. 3 hours), and at intervals of 7, 14 and 28 days after application. 10 g soil dw per replicate was extracted by adding 50 mL 1 M KCl solution to the equivalent of 10 g soil dw and mixing on a rotator at 150 rpm for 60 minutes. For the quantitative determination of the mineralized part of nitrogen the Autoanalyzer was used.
Statistics:	The mean nitrogen-content, standard deviation and coefficient of variation were calculated for each treatment group and sampling date.

Findings:

Table 9.5-4: Effects of Reg.No. 5079288 (RPA 407922) on nitrogen transformation

Sampling date (DAA)	Treatment	Measured values [mg NO_3-N/100 g sdw]	Mean value [mg NO_3-N/ 100 g sdw] (SD)	CV [%]	mg NO_3-N/ kg sdw/day	Deviation from control [%]
0	Control	2.61	2.64 (0.04)	1.7	-	-
		2.62				
		2.69				
	0.1 mg/kg sdw	2.68	2.63 (0.05)	1.9	-	-
		2.58				
		2.64				
	1.0 mg/kg sdw	2.61	2.61 (0.01)	0.2	-	-
		2.61				
		2.60				
7	Control	5.09	5.30 (0.23)	4.3	7.6	-
		5.54				
		5.26				
	0.1 mg/kg sdw	5.54	5.44 (0.09)	1.6	7.8	+5.8
		5.38				
		5.41				
	1.0 mg/kg sdw	5.29	5.27 (0.09)	1.7	7.5	+0.1
		5.34				
		5.17				
14	Control	5.63	5.85 (0.25)	4.3	4.2	-
		5.80				
		6.13				
	0.1 mg/kg sdw	6.20	6.08 (0.11)	1.7	4.3	+7.3
		6.04				
		6.00				
	1.0 mg/kg sdw	5.91	6.01 (0.09)	1.5	2.6	+6.0
		6.08				
		6.05				
28	Control	7.19	7.35 (0.14)	1.9	2.7	-
		7.47				
		7.38				
	0.1 mg/kg sdw	7.66	7.69 (0.23)	3.0	2.7	+7.5
		7.48				
		7.94				
	1.0 mg/kg sdw	7.61	7.48 (0.23)	3.1	2.7	+3.5
		7.62				

		7.21				

sdw…soil dry weight
DAA…Days after application
CV %...Coefficient of Variation in %
+…increase of nitrogen transformation; -…decrease of nitrogen transformation

In a separate study the reference item Dinoterb caused an effect on nitrogen transformation of +17.6%, +33.7% and + 42.6% at concentrations of 6.80, 16.00 and 27.00 mg dinoterb/kg soil dw, respectively, 28 days after application.

Conclusion: Reg.No. 5079288 caused no long-term adverse effects (difference to control < 25 %, OECD 216) on the soil nitrogen transformation (expressed as NO_3-N production) at the end of 28-day incubation period.

Comment RMS: The study was evaluated following the recommendations of the currently valid test guideline OECD 216 (2000).

Check of validity criteria:

- Evaluations of test results with agrochemicals are based on relatively small differences (i.e. average value ± 25 %) between nitrate concentrations in control and treated soil samples, so large variations in the controls can lead to false results. Therefore, the variation between replicate control samples should be less than ± 15%. In the current study the variation coefficient of replicate control samples ranged between 1.7 and 4.3% for the single sampling dates. The overall coefficient of variation for the control, estimated by ToxRat® 3.1.0., was 1.9%. Fulfilled.

Acceptability of the analytical methods used in the test: Evaluation of the analytical methods (water/sediment/soil) according to the Guidance Document SANCO/3029/99 rev. 4.

The chemicals for the calibration solutions were NANo2, (NH4)2SO4 and KNO3 from Merck. The autoanalyzer was calibrated before each measurement series by establishing a calibration curve. Each 30 samples a standard was measured for recalibrations and adjusting the calibration curve. The calibration curve was calculated with linear regression.

LOQ: 0.6 mg/ kg soil dw

LOD: not reported

The analytical method fulfils the requirements of guideline SANCO/3029/99 rev. 4.

Endpoints: Only the mean transformation rate over the replicates of one sampling was provided in the study report, transformation rates per replicate were not reported and could therefore not be recalculated by the RMS with ToxRat® 3.1.0. However, the data presented in the study report appears to be plausible and is considered acceptable.

	28 day EC_{25} > 1 mg test item/kg soil dw **Conclusion of the RMS:** Based on the evaluation of the study the nitrogen transformation test on soil microorganisms is considered valid.

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

According to the data requirements for active substances (Commission Regulation (EU) 283/2013) and/or plant protection products (Commission Regulation (EU) 284/2013) no testing on non-target terrestrial plants is necessary for seed treatments.

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

No studies regarding effects on other terrestrial organisms were submitted for the renewal of the EU approval of the active substance.

Based on the overall risk assessment no further studies on other terrestrial organisms are required.

B.9.8. EFFECTS ON BIOLOGICAL METHODS FOR SEWAGE TREATMENT

For the first EU approval of the active substance triticonazole a study on the inhibitory effect on the respiration of activated sewage sludge was submitted. For the current submission, this study has been re-evaluated. The study summary is provided below.

Reference:	**Assessment of the inhibitory effect on the respiration of activated sewage sludge**
Author(s), year:	Mead, C., 2000
Report/Doc. number:	C011667
Guideline(s):	OECD 209 (1984), OPPTS 850.6800
GLP:	Yes
Deviations:	Please refer to the commenting box below
Validity:	Acceptable

Material and methods:

Test substance:	triticonazole, batch no.: 9908001, purity: 911 g/kg
Test species:	Activated sludge microorganisms; aeration stage of the Severn Trent Water Plc sewage treatment plant, UK
Type of test, duration:	Laboratory aerobic activated sludge inhibition, limit test, 3 hours
Applied concentrations:	
Range finding test:	100 and 1000 mg/L
Definitive test:	Control, 1000 mg/L, 3 replicates
Toxic reference	3,5-dichlorophenol tested at concentrations of 3.2, and 32 mg/L
Substrate/test vessel:	Activated sludge (solid concentration: 4g/L) and synthetic sewage feed (according OECD guideline) treated with the test item (prepared by a direct dispersion in water), reference substance and inoculum control, final volume 500 mL/flask; pH:

7.6

Incubation: Continuous aeration at 21°C

Test parameters: Inhibition of respiration rate (oxygen consumption rates) after 30 minutes contact time and after 3 hours contact time was measured.

pH-values were determined at the test preparation at the end of the exposure period.

Statistics: The respiration rate was expressed as a percentage of the two control respiration rates.

Percentage inhibition was plotted against concentration for the reference material only and the EC_{50} values derived by inspection of the graph. The EC_{50} values for the test material were determined by inspection of the inhibition of respiration rate data.

Findings:

Range finding test: No significant effect on respiration was observed at either concentration employed

Definitive test:

Table 9.8-1: Effect of triticonazole on the respiration rate of activated sludge after 30 minutes contact time

Test substance	Nominal concentration [mg/L]	pH	Respiratory rate [mg O_2/L/min]	Oxygen concentration [mg O_2/L]		Inhibition [%]
				Start	End	
Control R1	-	8.0	0.47	7.4	2.7	-
Control R2	-	8.0	0.44	7.1	2.7	-
Triticonazole R1	1000	8.0	0.48	6.7	1.9	+5
Triticonazole R2	1000	7.9	0.49	6.0	1.6	+8
Triticonazole R3	1000	7.9	0.46	6.3	1.7	+1
Toxic reference	3.2	7.9	0.40	7.2	3.2	12
	10	8.1	0.28	7.8	5.0	38
	32	8.1	0.10	8.1	7.1	78

R1-R3…replicates 1-3
+ increase in respiration rate as compared to controls

Table 9.8-2: Effect of triticonazole on the respiration rate of activated sludge after 3 hours contact time

Test substance	Nominal concentration [mg/L]	pH	Respiratory rate [mg O_2/L/min]	Oxygen concentration [mg O_2/L]		Inhibition [%]
				Start	End	
Control R1	-	8.0	0.43	6.8	2.5	-
Control R2	-	8.0	0.45	7.5	3.0	-
Triticonazole R1	1000	8.0	0.42	7.4	3.2	5
Triticonazole R2	1000	7.9	0.45	7.3	2.8	+2
Triticonazole R3	1000	7.9	0.41	7.3	3.2	7
Toxic reference	3.2	7.9	0.31	6.6	3.5	30
	10	8.1	0.20	8.3	6.3	55
	32	8.1	0.06	8.2	7.6	86

R1-R3…replicates 1-3
+ increase in respiration rate as compared to controls

Conclusions: 3 hour EC_{50} > 1000 mg ai/L
3 hour NOEC = 1000 mg ai/L
based on nominal concentrations.

Comment RMS: The study was evaluated following the recommendations of the currently valid test guideline OECD 209 (2010).

Check of validity criteria:

- The blank controls (without the test substance or reference substance) oxygen uptake rate should not be less than 20 mg oxygen per one gram of activated sludge (dry weight of suspended solids) in an hour. If the rate is lower, the test should be repeated with washed activated sludge or with the sludge from another source. The coefficient of variation of oxygen uptake rate in control replicates should not be more than 30% at the end of definitive test. In the current test, the oxygen uptake rate is not given in mg oxygen per gram activated sludge. It is reported that the suspended solids in the inoculum were 4 g/l prior to use. It is assumed that this is the value for wet weight. Therefore the dry weight had to be 1.29 g/l to fulfil the validity criterion. It is not possible to assess the validity. The study was conducted according to an older guideline (OECD 209, 1984), fulfilling the validity criterion of this guideline (the two control respiration rates are within 15% of each other. In the current study 6.4%).
- In a 2004 international ring test organized by ISO using activated sludge derived from domestic sewage, the EC_{50} of 3,5-DCP was found to lie in the range 2 mg/L to 25 mg/L for total respiration, 5 mg/L to 40 mg/L for heterotrophic respiration and 0.1 mg/L to 10 mg/L for nitrification respiration. If the EC_{50} of 3,5-DCP does not lie in the expected range, the test should be repeated with activated sludge from another source. In the current study the 3 hours EC_{50} for the reference item was 8 mg/L. Fulfilled.

Acceptability of the analytical methods used in the test: According to the study report analysis of the concentration, homogeneity and stability of the test material in the test preparations was not appropriate to the test guideline.
No further information regarding the analytical methods was reported.

Endpoints: The RMS agrees on the endpoints given in the study report.
3 hour EC_{50} > 1000 mg ai/L
3 hour NOEC = 1000 mg ai/L

Conclusion of the RMS: It was not possible to assess the validity of the study. However, the validity criterion of the former OECD version (1984) was fulfilled.

B.9.9. MONITORING DATA

No monitoring data available

B.9.10. BIOLOGICAL ACTIVITY OF METABOLITES POTENTIALLY OCCURRING IN GROUNDWATER

There are no concerns for groundwater from the use of triticonazole in accordance with the use pattern for the current formulation.

PEC_{GW} values were calculated for the active substance and its metabolites RPA 404766, RPA 406341 and RPA 407922 considering the representative GAP uses. The PEC_{GW} were calculated using the model FOCUS PEARL and FOCUS PELMO and FOCUS MACRO. For details of the calculation please refer to Section B.8-CP

Based on the FOCUS modelling no PEC_{GW} values (for all FOCUS scenarios) greater than 0.1 µg/L were determined for the active substance and its metabolites considering all FOCUS scenarios.

Hence, no assessment of the biological activity of these metabolites is considered necessary.

B.9.11. REFERENCES RELIED ON

Reference:	**Literature search report final – Triticonazole – BASF confidential**
Author(s), year:	Zander-El-Metwally, M., Esswein, U., 2015
Report/Doc. number:	2015/1216973
Guideline(s):	EFSA (2011)
GLP:	Not applicable
Validity:	Acceptable

A literature search on triticonazole and the common product trade names was performed by the BASF Group Information Center. Three databases (CAPLUS, BIOSIS and CABA) were searched for ecotoxicology. The first step of the search result processing based on summary records involved the separation into "hits" and "ballast" (obviously irrelevant records). The “ballast” was not further processed.

The “hits” were further evaluated by the scientific experts and categorized into “not relevant”, “not reliable”, and “used for dossier”.

Duplicates of search results from different databases in a respective section were removed in STN databases by the “*duplicate remove*” command.

The search process is documented in all details with search profiles, search histories and summary tables according the GUIDANCE OF EFSA, Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) No 1107/2009, EFSA Journal 2011;9(2):2092.

The process of selection of relevant scientific peer-reviewed open literature was done in two steps:

The *First Selection step* for relevance based on summary records (e.g. titles, abstracts, index terms, keywords) was done by the Agro Information Professionals.

Obviously irrelevant records were tagged as “Ballast”. This ballast was controlled by scientific experts in the corresponding subject areas but was not further processed.

- Summary records which appear to be relevant and those of unclear relevance were tagged as “Hit” and went to the next level of evaluation.

The *Second Detailed Assessment* was done by the scientific experts in the corresponding areas.

Records tagged as “Hit” were further evaluated in depth.

To facilitate a comprehensible listing of the "Hits" in the different regulatory areas an Excel file was generated for each section with 3 typical registers, namely:

- "no relevant endpoint"
- "evaluated - not-relevant"
- "used for dossier"

In a first step (rapid assessment) the "Hits" were reviewed based on the information given in the **title and the abstract** with regard to relevance for the regulatory endpoints in the respective regulatory area. Those records which were clearly judged as not assignable to any regulatory endpoint were shifted into the register **"no relevant endpoint"** with an explaining reasoning.

In a second step (detailed assessment), all remaining records were assessed in detail based on the **complete report** by the respective expert(s) and separated into relevant reports for further discussion and those clearly not relevant.

Criteria to assign a record to the register **"evaluated - not-relevant"** were:

- Those records which provided information supporting the existing regulatory data package without any new relevant data or information were classified as "confirmatory data"
- Those records which were not assignable to the substance of interest (for example mixtures, not about test substance or other relevant substance)
- Secondary literature linking to primary literature already discussed under relevant records
- and those which were judged as not relevant due to other reasons with a respective justification.

Criteria to assign a record to the register **"used for dossier"** were:

- Records providing information about additional/new/unknown/potentially contradictory effects or data which might impact the hazard assessment endpoints or the risk assessments parameters and which in addition have a high grade of reliability of grade 1 or 2 based on the 'Klimisch' scoring system (see below).

Those records assigned to the category "used for dossier" were provided with a Doc ID and discussed in detail in the respective dossier chapter.

Reliability scoring system based on Klimisch et al., 1997:

Reliability 1: **reliable without restrictions:** studies or data generated according to generally valid and/or internationally accepted testing guidelines (preferably performed according to GLP) or in which the test parameters documented are based on a specific (national) testing guideline or in

which all parameters described are closely related/comparable to a guideline method. (e.g. literature about toxicity / ecotoxicity study consistent with requests of international testing guidelines and performed under GLP conditions with experienced and trained personal)

Reliability 2: **reliable with restrictions:** studies or data (mostly not performed according to GLP), in which the test parameters documented do not totally comply with the specific testing guideline, but are sufficient to accept the data or in which investigations are described which cannot be subsumed under a testing guideline, but which are nevertheless well documented and scientifically acceptable (appropriately documented studies which meets basic scientific principles, mechanistic studies)

Reliability 3: **not reliable:** studies or data in which there were interferences between the measuring system and the test substance or in which organisms/test systems were used which are not relevant in relation to the exposure (e.g. unphysiologic pathways of application) or which were carried out or generated according to a method which is not acceptable, the documentation of which is not sufficient for assessment and which is not convincing for an expert judgement (e.g. literature studies with insufficient information or according to unvalidated method)

Reliability 4: **not assignable:** studies or data which do not give sufficient experimental details and which are only listed in short abstracts or secondary literature

The RMS concluded that appropriate time frame, databases, (extensive) keywords and evaluation criteria were applied, all this according to EFSA Guidance on submission of scientific peer-reviewed open literature. For the articles which were assigned as relevant and reliable the notifier provided in-depth robust study summaries. The evaluations are included in the respective chapters.

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 8.1.1.1	[redacted]	1991a	RPA 400727: Acute Oral Toxicity (LD_{50}) to Bobwhite Quail [redacted] Document No: R013025 GLP Yes unpublished	Y	N	-	BASF	In the DAR (2003)
KCA 8.1.1.1	[redacted]	1991b	RPA 400727: Acute Oral Toxicity (LD_{50}) to Mallard Duck [redacted] Document No: R013024 GLP Yes unpublished	Y	N	-	BASF	In the DAR (2003)
KCA 8.1.1.1	[redacted]	1992a	RPA 400727 - Acute Oral Toxicity (LD_{50}) to Grey Partridge [redacted]; Document No: R013069 GLP Yes unpublished	Y	N	-	BASF	In the DAR (2003)
KCA 8.1.1.1	[redacted]	1992b	Acute Oral toxicity (LD_{50}) to Red-Legged Partridge [redacted] Document No: R013065 GLP Yes unpublished	Y	N	-	BASF	In the DAR (2003)
KCA 8.1.1.1	[redacted]	1990a	The Acute Oral Toxicity of RPA 400-727 to the Pigeon [redacted] Document No: R013005 GLP No unpublished	Y	N	-	BASF	In the DAR (2003)
KCA 8.1.1.1	[redacted]	1990b	The Acute Oral Toxicity of RPA 400-727 to the Ring-Necked Pheasant [redacted] Document No: R013004 GLP No unpublished	Y	N	-	BASF	In the DAR (2003)
KCA 8.1.1.1	[redacted]	2000a	RPA 406341 An Acute Oral Toxicity Study with the Northern Bobwhite: RPA 406341 [redacted] Document No: B002787 GLP Yes unpublished	Y	N	-	BASF	In the DAR (2003)
KCA 8.1.2.1	[redacted]	1992a	RPA 400727: Subacute dietary Toxicity (LC_{50}) to Bobwhite Quail [redacted]; Document No: R013037 GLP Yes unpublished	Y	N	-	BASF	In the DAR (2003)
KCA	[redacted]	1992b	RPA 400727: Subacute dietary Toxicity	Y	N	-	BASF	In the DAR

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
8.1.2.1	[redacted]		(LC_{50}) to Mallard Duck [redacted] Document No: R013038 GLP Yes unpublished					(2003)
KCA 8.1.3	[redacted]	1995a	The Reproduction Toxicity Test of RPA400727 in Northern Bobwhite (*Colinus virginianus*) [redacted] Document No: R013161 GLP Yes unpublished	Y	N	-	BASF	In the DAR (2003)
KCA 8.1.3	[redacted]	1998b	The Reproductive Toxicity Test of RPA400727 with the Mallard Duck (*Anas platyhynchos*) [redacted] Document No: R000098 GLP Yes unpublished	Y	N	-	BASF	In the DAR (2003)
KCA 8.1.3	[redacted]	2003	Triticonazole (BAS 595 F): The Reproductive Toxicity Test of RPA400727 with the Mallard duck (Anas platyhrynchos); [redacted] 1998 BASF DocID 2003/1006403	Y	N	-	BASF	In the DAR (2003)
KCA 8.1.1.3	Ott K., Pascual J., Kragten S., Ristau K.	2016	Triticonazole (BAS 595F) – Position Paper on the Preliminary Birds and mammals Risk Assessment by AGES for the EU AIR III Renewal of Triticonazole BASF DocID 2016/1321104 GLP No unpublished	Y	Y	New data for AIR 3 renewal	BASF	Submitted for the purpose of renewal (2015)
KCA 8.1.1.3	[redacted]	2012a	BAS 595F (Triticonazole) – 1-Generation –Reproduction Study on the Bobwhite quail (*Colinus virginianus*) by Administration in the Diet BASF DocID 2011/1269059 [redacted]. GLP Yes Unpublished	Y	Y	New data for AIR 3 renewal	BASF	Submitted for the purpose of renewal (2015)
KCA 8.1.4	[redacted]	2007	BAS 595 F – 1-Generation Reproduction Study on the Bobwhite (Colinus Virginianus) by Administration in the Diet BASF DocID 2006/1026908 [redacted] GLP Yes Unpublished	Y	N	-	BASF	As confirmatory Data (2009) for the first approval (2003)
KCA 8.1.4	[redacted]	2008	BAS 595 F (Triticonazole),1-Generation Reproduction Study on the Bobwhite (*Colinus Virginianus*) by Administration in the Diet for Four Weeks BASF DocID 2008/1023059 [redacted]	Y	N	-	BASF	As confirmatory Data (2009) for the first approval (2003)

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
			[redacted] GLP Yes Unpublished					
KCA 8.2.1.	[redacted]	1990a	The acute Toxicity of RPA 400727 to Rainbow Trout (*Oncorhynchus mykiss*) [redacted] Document No: R013008 GLP Yes unpublished	Y	N	-	BASF	In the DAR (2003)
KCA 8.2.1.	[redacted]	1998a	Acute toxicity (96 hours) to Rainbow Trout (*Oncorhynchus mykiss*) under Flow-Through Conditions Triticonazole [redacted] Document No: C017670 GLP Yes unpublished	Y	N	-	BASF	In the DAR (2003)
KCA 8.2.1.	[redacted]	1998b	RPA 400727 – Acute Toxicity to Bluegill Sunfish (*Lepomis macrochirus*) Under Flow-Through Conditions [redacted] Document No: R012019 GLP Yes unpublished	Y	N	-	BASF	In the DAR (2003)
KCA 8.2.1.	[redacted]	2006a	BAS 595 F Acute Toxicity Study on the Rainbow Trout (*Oncorhynchus mykiss*) in a static system over 96 hours BASF DocID 2006/1015993 [redacted] GLP Yes Unpublished	Y	Y	New data for AIR 3 renewal	BASF	Submitted for the purpose of renewal (2015)
KCA 8.2.1.	[redacted]	2006b	BAS 595 F Acute Toxicity Study on the Bluegill sunfish (*Lepomis macrochirus*) in a static system over 96 hours BASF DocID2006/1018146 [redacted] GLP Yes Unpublished	Y	Y	New data for AIR 3 renewal	BASF	Submitted for the purpose of renewal (2015)
KCA 8.2.1.	[redacted]	1998a	Triticonazole technical - Acute toxicity to Sheepshead Minnow (*Cyprinodon variegatus*) under flow-through conditions Document No: R000095 [redacted] GLP Yes Unpublished	Y	N	-	BASF	Submitted for the purpose of renewal (2015)
KCA 8.2.1.	[redacted]	2014a	BAS 595 F (Triticonazole) Acute Toxicity Study in the Common Carp (*Cyprinus carpio*) BASF DocID 2014/1095638 [redacted] GLP Yes Unpublished	Y	Y	New data for AIR 3 renewal	BASF	Submitted for the purpose of renewal (2015)
KCA	Durjava M.K.	2013a	Experimental assessment of the	N	N	-	Public	Submitted

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
8.2.1 and KCA 8.2.4.1	et al.		environmental fate and effects of Triazoles and Benzotriazole BASF DocID 2015/1177633 GLP No Published					for the purpose of renewal (2015)
KCA 8.2.2.1	[redacted]	1996a	Triticonazole: Fish, Juvenile Growth Test – 28 Days [redacted] Document No: R013166 GLP Yes unpublished	Y	N	-	BASF	In the DAR (2003)
KCA 8.2.2.1	[redacted]	1998b	Triticonazole technical: Early life stage toxicity test with Fathead minnow (*Pimephales promelas*) Document No: B003479 [redacted] GLP Yes Unpublished	Y	N	-	BASF	Submitted for the purpose of renewal (2015)
KCA 8.2.2.1	[redacted]	1998c	Triticonazole technical: Early life stage toxicity test with Fathead minnow (*Pimephales promelas*) Document No: C044319 [redacted] GLP Yes Unpublished	Y	N	-	BASF	Submitted for the purpose of renewal (2015)
KCA 8.2.2.1	[redacted]	2006a	BASF 595F - Early life stage toxicity test with sheepshead minnow (*Cyprinodon variegatus*) BASF DocID 2006/7007245 [redacted] GLP Yes Unpublished	Y	Y	New data for AIR 3 renewal	BASF	Submitted for the purpose of renewal (2015)
KCA 8.2.2.2	[redacted]	2008a	BAS 595 F (Triticonazol) - Life cycle test on the fathead minnow (*Pimephales promelas*) in a flow through system BASF DocID: 2008/1028361 [redacted] GLP Yes Unpublished	Y	Y	New data for AIR 3 renewal	BASF	Submitted for the purpose of renewal (2015)
KCA 8.2.2.2	[redacted]	2012a	BAS 595 F (Triticonazol) - Life cycle toxicity test on the fathead minnow (*Pimephales promelas*) in a flow through system BASF DocID 2012/1079000 [redacted] GLP Yes Unpublished	Y	Y	New data for AIR 3 renewal	BASF	Submitted for the purpose of renewal (2015)
KCA 8.2.3.1	[redacted]	1994a	A Fish Bioaccumulation and Depuration Study on RPA 400727 [redacted] Document No: R013103 GLP Yes unpublished	Y	N	-	BASF	In the DAR (2003)
KCA	[redacted]	1996a	Bioaccumulation Test in Bluegill	Y	N	-	BASF	In the DAR

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
8.2.3.1	[redacted]		Sunfish (14C)-Triticonazole [redacted] Document No: C017724 GLP Yes unpublished					(2003)
KCA 8.2.3.1	[redacted]	1999	Bioaccumulation Test in Bluegill Sunfish (1. Amendment) [14C]-Triticonazole [redacted] Document No: R005940 GLP Yes unpublished	Y	N	-	BASF	In the DAR (2003)
KCA 8.2.4.1.	Douglas M.T., Halls R.W.S., Macdonald I.A.	1990b	The Acute Toxicity of RPA 400727 to *Daphnia magna* Generated by: Rhône Poulenc; Huntington Research Centre Ltd., Huntington, England Document No: R013007 GLP Yes unpublished	N	N	-	BASF	In the DAR (2003)
KCA 8.2.4.1.2	Sewell I.G., Mullee D.M.	2001a	Acute Toxicity to *Daphnia magna* RPA 407922 Generated by: Aventis CropScience GmbH, DEU; Ecotoxicology, Frankfurt Document No: C017901 GLP Yes unpublished	N	N	-	BASF	In the DAR (2003)
KCA 8.2.4.1	Sewell I.G., Mullee D.M.	2001b	Acute Toxicity to *Daphnia magna* RPA 404766 Generated by: Aventis CropScience GmbH, DEU; Ecotoxicology, Frankfurt Document No: C017902 GLP Yes unpublished	N	N	-	BASF	In the DAR (2003)
KCA 8.2.4.1	Sewell I.G., Mullee D.M.	2002a	RPA406341: Acute Toxicity to *Daphnia magna* Generated by: Aventis CropScience GmbH, DEU; Ecotoxicology, Frankfurt Document No: C020498 GLP Yes unpublished	N	N	-	BASF	In the DAR (2003)
KCA 8.2.4.1	Putt A.E.	1998a	RPA 406203 - Acute toxicity to daphnids (*Daphnia magna*) under flow-through conditions Document No: C044320 Springborn Laboratories Inc., Wareham MA, United States of America GLP Yes Unpublished	N	N	-	BASF	Submitted for the purpose of renewal (2015)
KCA 8.2.4.1	Janson G.-M.	2009a	Acute toxicity of Reg.No. 5079359 (metabolite of BAS 595 F) to *Daphnia magna* STRAUS in a 48 hour static test BASF DocID 2009/1075083 BASF SE, Limburgerhof, Germany Fed.Rep. GLP Yes Unpublished	N	Y	New data for AIR 3 renewal	BASF	Submitted for the purpose of renewal (2015)
KCA 8.2.4.2	Sousa J.V.	1998d	Triticonazole technical - Acute toxicity to mysids (*Mysidopsis bahia*) under	N	N	-	BASF	Submitted for the

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			flow-through conditions Document No: B004421 Springborn Laboratories Inc., Wareham MA, United States of America GLP Yes Unpublished					purpose of renewal (2015)
KCA 8.2.4.2	Dionne E.	1998a	Triticonazole technical - Acute toxicity to eastern oyster (*Crassostrea virginica*) under flow-through conditions Document No: C019777 Springborn Laboratories Inc., Wareham MA, United States of America GLP Yes Unpublished	N	N	-	BASF	Submitted for the purpose of renewal (2015)
KCA 8.2.5.1	Douglas M.T., Halls W.S., Anderson A.	1992a	An Assessment of the Effects of RPA 400727 on the Reproduction of *Daphnia magna* (Straus) Generated by: Rhône Poulenc; Huntington Research Centre Ltd., Huntington, England Document No: R013032 GLP Yes unpublished	N	N	-	BASF	Submitted for the purpose of renewal (2015)
KCA 8.2.5.1/1	Putt A.E.	2006a	BAS 595 F (Triticonazole) - Full life-cycle toxicity test with water fleas, *Daphnia magna*, under static-renewal conditions BASF DocID 2006/7007209 Springborn Smithers Laboratories, Wareham MA, United States of America GLP Yes Unpublished	N	Y	-	BASF	Submitted for the purpose of renewal (2015)
KCA 8.2.5.1	Urann K.	2012a	BAS 595 F - Full life cycle toxicity test with water fleas (*Daphnia magna*) under static-renewal conditions, following OPPTS draft guideline 850.1300 BASF DocID 2012/7003660 Springborn Smithers Laboratories, Wareham MA, United States of America GLP Yes Unpublished	N	Y	-	BASF	Submitted for the purpose of renewal (2015)
KCA 8.2.5.1	McElligott A.	1998a	Triticonazole - *Daphnia magna* life cycle (21-day static renewal) chronic toxicity study Document No: R013169 Rhone-Poulenc Agrochimie, Sophia Antipolis, France GLP Yes Unpublished	N	N	-	BASF	Submitted for the purpose of renewal (2015)
KCA 8.2.5.2	Putt A.E.	2006b	BAS 595 F (Triticonazole) - Life-cycle toxicity test with Mysids (Americamysis bahia) BASF DocID 2006/7007246 Springborn Smithers Laboratories, Wareham MA, United States of America GLP Yes Unpublished	N	Y	New data for AIR 3 renewal	BASF	Submitted for the purpose of renewal (2015)
KCA 8.2.6	Handley J.W., Mead C., Bartlett A.J.	1992a	Assessment of the Algistatic Effect of RPA 400727 to *Selenastrum capricornutum* Generated by: Safepharm Laboratories Limited, Derby, England; Rhône	N	N	-	BASF	In the DAR (2003)

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			Poulenc Agrochimie, Lyon, France; Document No: R013056 GLP Yes unpublished					
KCA 8.2.6	Hoberg J.R.	1998a	Triticonazole – Toxicity to the Freshwater Blue-Green Alga – *Anabaena flos aquae* Generated by: Springborn Laboratories, Inc., Wareham, USA Document No: R012015 GLP Yes unpublished	N	N	-	BASF	In the DAR (2003)
KCA 8.2.6	Hoberg J.R.	1998b	Triticonazole – Toxicity to the Duckweed, Lemna gibba Generated by: Springborn Laboratories, Inc. Wareham, USA Document No: R012023 GLP Yes unpublished	N	N	-	BASF	In the DAR (2003)
KCA 8.2.6.1	Hoberg J.R.	1998c	Triticonazole - Toxicity to the freshwater green alga, *Selenastrum capricornutum* Document No: R012017 Springborn Laboratories Inc., Wareham MA, United States of America GLP Yes Unpublished	N	N	-	BASF	Submitted for the purpose of renewal (2015)
KCA 8.2.6.1	Seeland-Fremer A., Wydra V.	2014a	Toxicity of Reg.No. 4378513 (Triticonazole technical (BAS 595 F)) to *Pseudokirchneriella subcapitata* in an algal growth inhibition test BASF DocID 2014/1083347 Institut fuer Biologische Analytik und Consulting IBACON GmbH, Rossdorf, Germany Fed.Rep. GLP Yes Unpublished	N	Y	New data for AIR 3 renewal	BASF	Submitted for the purpose of renewal (2015)
KCA 8.2.6.1	Hoffmann F.	2009a	Effect of Reg.No. 5079359 (metabolite of BAS 595 F, Triticonazole) on the growth of the green alga *Pseudokirchneriella subcapitata* BASF DocID 2009/1050280 BASF SE, Limburgerhof, Germany Fed.Rep. GLP Yes Unpublished	N	Y	New data for AIR 3 renewal	BASF	Submitted for the purpose of renewal (2015)
KCA 8.2.6.1	Peters A.	1992a	Toxicity of [redacted] to the *Scenedesmus subspicatus* CHODAT (96 hours) (according to guideline OECD No. 201 from June 1984) Document No: C039712 Oekolimna - Gesellschaft fuer Oekologie und Gewaesserkunde mbH, Burgwedel, Germany Fed.Rep. GLP Yes Unpublished	N	N	-	BASF	Submitted for the purpose of renewal (2015)
KCA 8.2.6.2	Hoberg J.R.	1998d	Triticonazole - Toxicity to the freshwater diatom, *Navicula pelliculosa* Document No: R012969 Springborn Laboratories Inc., Wareham MA, United States of America GLP Yes Unpublished	N	N	-	BASF	Submitted for the purpose of renewal (2015)
KCA	Hoberg J.R.	1998e	Triticonazole - Toxcity to the marine	N	N	-	BASF	Submitted

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8.2.6.2			diatom, *Skeletonema costatum* Document No: B004429 Springborn Laboratories Inc., Wareham MA, United States of America GLP Yes Unpublished					for the purpose of renewal (2015)
KCA 8.2.7.2	Van der Kolk J.	1998a	Triticonazole: Chronic Effects on Midge Larvae (*Chironomus riparius*) in a water/sediment system Generated by: Rhône Poulenc; Springborn Laboratories (Europe) AG, CHE; Document No: R005755 GLP Yes unpublished	N	N	-	BASF	In the DAR (2003)
KCA 8.3.1.1	Schmitzer S.	1998	Laboratory testing for toxicity (acute contact and oral LD_{50}), triticonazole to honey bees (*Apis mellifera* L.) (Hymenoptera, Apidae) Generated by: Rhone-Poulenc; IBACON, Rossdorf, Germany; Document No: R005760 GLP Yes unpublished	N	N	-	BASF	In the DAR (2003)
KCA 8.3.1.1.1 and 8.3.1.1.2	Hernadi D.	2006a	Effects of BAS 595 F (acute contact and oral) on honey bees (*Apis mellifera* L.) in the laboratory BASF DocID 2006/1024251 LAB International Research Centre Hungary Ltd., Veszprem, Hungary GLP Yes Unpublished	N	Y	New data for AIR 3 renewal	BASF	Submitted for the purpose of renewal (2015)
KCA 8.4.1	Handley J.W., Wetton P.M.	1991	The Acute Toxicity of RPA 400727 to Earthworms (*Eisenia foetida*) Generated by: Rhône-Poulenc; Safepharm Laboratories Limited, Derby, England; Document No: R013015 GLP / GEP Yes unpublished	N	N	-	BASF	In the DAR (2003)
KCA 8.4.1	Lührs U.	2002a	Acute toxicity (14 days) of RPA406341 to the earthworm Eisenia fetida in artificial soil Generated by: Aventis CropScience GmbH, DEU; IBACON GmbH, Rossdorf, DEU; Document No: C020497 GLP Yes unpublished	N	N	-	BASF	In the DAR (2003)
KCA 8.4.1	Lührs U.	2001	Acute toxicity (14 days) of RPA404766 to the earthworms *Eisenia fetida* in artificial soil Generated by: Aventis CropScience GmbH, DEU; IBACON GmbH, Rossdorf, DEU; Document No: C017900 GLP Yes unpublished	N	N	-	BASF	In the DAR (2003)
KCA 8.4.1	Lührs U.	2002b	Acute toxicity (14 days) of RPA407922 to the earthworm *Eisenia fetida* in artificial soil Generated by: Aventis CropScience GmbH, DEU; IBACON GmbH, Rossdorf, DEU; Document No: C021833	N	N	-	BASF	In the DAR (2003)

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			GLP Yes unpublished					
KCA 8.4.2	Lührs U.	1999	Effects of Triticonazole on Reproduction and Growth of Earthworms *Eisenia fetida* (Savingy 1826) in Artifical Soil Generated by: Inst. für Biologische Analytik, Rossdorf, Germany; Rhône-Poulenc Agro, Sophia-Antipolis, France; Document No: R006093 GLP Yes unpublished	N	N	-	BASF	In the DAR (2003)
KCA 8.4.1	Friedrich S.	2013a	Sublethal toxicity of Reg.No. 5079285 (metabolite of BAS 595 F, Triticonazole) to the earthworm *Eisenia fetida* in artificial soil BASF DocID 2014/1000026 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. GLP Yes Unpublished	N	Y	New data for AIR 3 renewal	BASF	Submitted for the purpose of renewal (2015)
KCA 8.4.1	Friedrich S.	2013b	Sublethal toxicity of Reg.No. 5079288 (metabolite of BAS 595 F, Triticonazole) to the earthworm *Eisenia fetida* in artificial soil BASF DocID 2014/1000027 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. GLP Yes Unpublished	N	Y	New data for AIR 3 renewal	BASF	Submitted for the purpose of renewal (2015)
KCA 8.4.1	Wolf A.	2006a	Effects of Reg.No. 5059144 (RPA 406341, metabolite of BAS 595 F) on growth and reproduction of earthworms (*Eisenia fetida*) in artificial soil with 5% peat BASF DocID 2006/1030247 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. yes Unpublished	N	Y	New data for AIR 3 renewal	BASF	Submitted for the purpose of renewal (2015)
KCA 8.4.2.1	Friedrich S.	2013c	Effects of BAS 595 F (Triticonazole) on the reproduction of the collembolan *Folsomia candida* BASF DocID 2014/1000021 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. GLP Yes Unpublished	N	Y	New data for AIR 3 renewal	BASF	Submitted for the purpose of renewal (2015)
KCA 8.4.2.1	Schulz L.	2014a	Effects of BAS 595 F (Triticonazole) on the reproduction of the predatory mite *Hypoaspis aculeifer* BASF DocID 2014/1000022 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. GLP Yes Unpublished	N	Y	New data for AIR 3 renewal	BASF	Submitted for the purpose of renewal (2015)
KCA 8.4.2.1	Friedrich S.	2013d	Effects of Reg.No. 5079285 (metabolite of BAS 595 F, Triticonazole) on the reproduction of the collembolan *Folsomia candida*	N	Y	New data for AIR 3 renewal	BASF	Submitted for the purpose of renewal

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			BASF DocID 2014/1000028 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. GLP Yes Unpublished					(2015)
KCA 8.4.2.1	Royer S.	2006a	Effect of Reg.No. 5059144 (RPA 406341, metabolite of BAS 595 F) on the reproduction of the collembola *Folsomia candida* in artificial soil with 5% peat BASF DocID 2006/1031679 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. GLP Yes Unpublished	N	Y	New data for AIR 3 renewal	BASF	Submitted for the purpose of renewal (2015)
KCA 8.4.2.1	Ganssmann M.	2014a	Effect of Reg. No. 5059144 (metabolite of BAS 595 F) on reproduction of the predatory mite *Hypoaspis aculeifer* in artificial soil with 5% peat BASF DocID 2014/1083348 Institut fuer Biologische Analytik und Consulting IBACON GmbH, Rossdorf, Germany Fed.Rep. GLP Yes Unpublished	N	Y	New data for AIR 3 renewal	BASF	Submitted for the purpose of renewal (2015)
KCA 8.4.2.1	Friedrich S.	2013e	Effects of Reg.No. 5079288 (metabolite of BAS 595 F, Triticonazole) on the reproduction of the collembolan *Folsomia candida* BASF DocID 2014/1000029 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. yes Unpublished	N	Y	New data for AIR 3 renewal	BASF	Submitted for the purpose of renewal (2015)
KCA 8.5.1	Aldred D., Seal K.J.	1991	A laboratory assessment of the effects of RPA 400727 on Soil microflora respiration and nitrogen turnover Generated by: Rhône-Poulenc; Euro Laboratories Limited, Cranfield, England; Document No: R013039 GLP / GEP Yes unpublished	N	N	-	BASF	In the DAR (2003)
KCA 8.5.1	Leake C.R.	1998	A laboratory assessment of the effects of RPA 400727 on Soil microflora respiration and nitrogen turnover - amendment Generated by: Rhône-Poulenc; Euro Laboratories Limited, Cranfield, England; Document No: C017774 GLP / GEP Yes unpublished	N	N	-	BASF	In the DAR (2003)
KCA 8.5	Royer S.	2006b	Effect of Reg.No. 5059144 (RPA 406341, metabolite of BAS 595 F) on soil microorganisms: Nitrogen transformation test BASF DocID 2006/1028713 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. GLP Yes Unpublished	N	Y	New data for AIR 3 renewal	BASF	Submitted for the purpose of renewal (2015)
KCA	Stojanowitsch	2015a	Reg.No. 5079285 (metabolite of BAS	N	Y	New data for	BASF	Submitted

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8.5	M.		595 F, Triticonazole): Effects on the activity of the soil microflora under laboratory conditions (nitrogen transformation) BASF DocID 2013/1103636 BASF SE, Limburgerhof, Germany Fed.Rep. GLP Yes Unpublished			AIR 3 renewal		for the purpose of renewal (2015)
KCA 8.5	Schulz L.	2014b	Effects of Reg.No. 5079288 (metabolite of BAS 595 F, Triticonazole) on the activity of soil microflora (Nitrogen transformation test) 2014/1000025 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. GLP Yes Unpublished	N	Y	New data for AIR 3 renewal	BASF	Submitted for the purpose of renewal (2015)
KCA 8.7	Royer S.	2006c	Effect of Reg.No. 5059144 (RPA 406341, metabolite of BAS 595 F) on soil microorganisms: Carbon transformation test 2006/1028714 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. GLP Yes Unpublished	N	Y	New data for AIR 3 renewal	BASF	Submitted for the purpose of renewal (2015)
KCA 8.7	Niewiadomska A., Sawińska Z., Wolna-Maruwka A.,	2011	Impact of selected seed dressings on soil microbiological activity in spring barley cultivation Fresenius Environmental Bulletin, 20(5A):1252-1261, pp. 1252-1261 GLP No published	N	N	-	Public	Submitted for the purpose of renewal (2015)
KCA 8.7	Mead C.	2000	Assessment of the inhibitory effect on the respiration of activated sewage sludge Triticonazole Generated by: Safepharm Laboratories Limited, Derby, GBR; Aventis CropScience GmbH, DEU; Ecotoxicology, Frankfurt Document No: C011667 GLP Yes unpublished	N	N	-	BASF	In the DAR (2003)