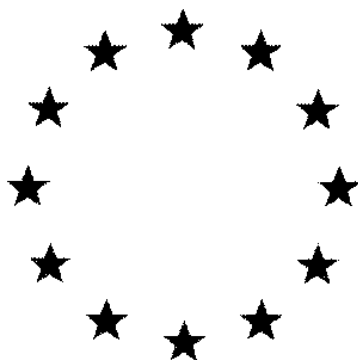


European Commission



**Draft (Renewal) Assessment Report prepared
according to the Commission Regulation (EC) No
1107/2009**

**Daminozide (ISO); 4-(2,2-
dimethylhydrazino)-4-oxobutanoic
acid; *N*-dimethylaminosuccinamic
acid**

Volume 3 – B.9 (PPP) – Dazide Enhance

Rapporteur Member State: Czech Republic
Co-Rapporteur Member State: Hungary

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B.9 Ecotoxicology

This document is a summary of the information presented for this section for the first inclusion and for the purpose of AIR 3.

A risk assessment for non-target organisms is presented for Daminozide (ISO); 4-(2,2-dimethylhydrazino)-4-oxobutanoic acid; N-dimethylaminosuccinamic acid ('hereafter referred to as 'daminozide') in the Dazide Enhance formulation (code FAL 2400), synonymous with Dazide 85 WG, Dazide WG, Dazide SG. Dazide Enhance is a water soluble granule formulation (SG) containing 850 g/kg daminozide. The product is a plant growth regulator intended for use on field and protected ornamental plants. The mode of action is through interference with gibberellic acid biosynthesis. It is absorbed by the leaves and translocated throughout the treated plant. As a result more compact plants (by inhibition of intermodal elongation) are produced.

Intended application pattern

The use pattern for this formulation is summarised in Table B.9-1.

Table B. 9- 1: Intended application pattern

Crop	Timing of application BBCH	Method of application	Number of applications	Interval between applications (min.)	Maximum application rate, individual treatment	
					Product [kg/ha]	Daminozide [kg a.s./ha]
Ornamentals (Protected)	<50	Over spray (Gantry)	1 - 5	7 days	9	7.65
Ornamentals (Field)	<50	Foliar*	1 - 5	7 days	5	4.25

* Application using a knapsack sprayer

It is not stated in the GAP, that the protected use is restricted to permanent greenhouses only. Based on Technical report on the outcome of the pesticides peer review meeting on general recurring issues in ecotoxicology (EFSA, 2015), the risk assessment for birds, mammals, bees, non-target arthropods and non-target plants should be performed assuming the same exposure as for a field use, unless it is indicated that the uses will be restricted to permanent greenhouses.

Besides the formulation Dazide Enhance (FAL 2400), another formulation Alar (B-Nine, UBI 6899-00 SP) is considered as the representative one for this renewal. Representative formulations used for Annex I inclusion were Dazide 85 (SP) and Alar 85 (UBI 2231-01 SP), the earlier formulations of the current ones. The differences in composition among all these formulations are considered as minor and their toxicities are considered to be comparable. For detailed composition of all these formulations see Volume 4 Annex C.

B.9.1 Effects on birds and other terrestrial vertebrates

B.9.1.1 Effects on birds

Summary of studies on toxicity to birds

The results of avian toxicity studies for daminozide are summarised in the table below.

Table B 9.1.1-1 Summary of avian toxicity studies for daminozide

Test species	Test substance	Test system	Endpoint	Toxicity (mg/kg bw/day)	Reference
Bobwhite quail (<i>Colinus virginianus</i>) ^{# 2}	Daminozide	Acute, oral 14 d	LD ₅₀	>2250 mg/kg bw* >4248 mg/kg bw³	██████████ (2006) 429-104
Mallard duck (<i>Anas platyrhynchos</i>) [#]	Daminozide	Acute, oral 14 d	LD ₅₀	>2250 mg/kg bw* >4248 mg/kg bw³	██████████ (1992) A.7.4.2.9
Bobwhite quail (<i>Colinus virginianus</i>) ^{# 1}	Daminozide	Short-term dietary, 5 day feeding	LC ₅₀ LDD ₅₀	n.a.	██████████ (1977) A.7.4.2.4
Mallard duck (<i>Anas platyrhynchos</i>) ^{# 1}	Daminozide	Short-term dietary, 5 day feeding	LC ₅₀ LDD ₅₀	n.a.	██████████ (1974) A.7.4.2.5
Bobwhite quail (<i>Colinus virginianus</i>) ^{# 1,4}	Alar 85	Short-term dietary, 5 day feeding	LC ₅₀ LDD ₅₀	n.a.	██████████ (1966a) A.7.4.2.1
Bobwhite quail (<i>Colinus virginianus</i>)	Daminozide	Subchronic and reproductive, 21 weeks feeding	NOEC NOEL	1000 ppm* 79.7 mg/kg bw/d*	██████████ (2012) 616-104

[#] Study evaluated in old DAR (1999).

* Maximum dose tested.

¹ Study is not considered valid

² A limit test.

³ Extrapolated from the reported endpoint of >2250 mg a.s./kg bw, based on a factor of 1.888

⁴ Study summarized and evaluated in Volume 3 CP B.9 for Alar

Endpoints used in the regulatory risk assessment included in bold.

Since the acute oral toxicity study with bobwhite quail is a limit test and no mortality was observed at a limit dose >2250 mg/kg, which tested 10 individuals, an extrapolation factor of 1.888 can be applied to the acute endpoints of >2250 mg a.s./kg bw in accordance with the EFSA Guidance on risk assessment for birds and mammals (2009), resulting in LD₅₀ value of **4248 mg a.s./kg bw** for birds.

Regarding the other acute toxicity study carried out with mallard duck, similarly no mortality and no effects on body weight and food consumption were observed at any dose tested, including the highest dose of 2250 mg/kg. Therefore, the extrapolation factor of 1.888 can also be applied to this acute endpoint and it is justified to use the extrapolated LD₅₀ value of **4248 mg a.s./kg bw** in acute risk assessment for birds.

B.9.1.2 Effects on terrestrial vertebrates other than birds**Summary of studies on toxicity to terrestrial vertebrates other than birds**

A summary of the key mammalian toxicity studies relevant to the ecotoxicological risk assessment is given in the table below. These data were evaluated in Section B.6 where further discussion can be found.

Table B 9.1.2-1 Summary of mammalian toxicity studies for daminozide

Substance	Species	Type of study, dose range tested	Study endpoint	Value, effects	Reference
Acute oral toxicity					
Daminozide	Rat	Acute oral, OECD 423, 5000 mg/kg bw	LD ₅₀	>5000 mg/kg bw	■■■■■ (1994)
Dazide Enhance	Rat	Acute oral, OECD 423, 5000 mg/kg bw	LD ₅₀	>5000 mg form./kg bw >4250 mg a.s./kg bw	■■■■■ (2003a)
B-Nine	Rat	Acute oral, OECD 423, 5000 mg/kg bw	LD ₅₀	>5000 mg form./kg bw >4250 mg a.s./kg bw	■■■■■ (1997a)
Short-term toxicity					
Daminozide	Rat	90-day (gavage), OECD 408, 40, 200, 1000 mg/kg bw/d	NOAEL	1000 mg/kg bw/d	■■■■■ 2005
Long-term toxicity					
Daminozide	Rat	Two-generation reproduction, OECD 416, 0, 5, 50 and 500 mg/kg bw/day (0, 100, 1000 and 10000 ppm)	NOEL (NOEC)	Parental: 50 mg/kg bw/d (1000 ppm) changes in food consumption and body weight Developmental: 500 mg/kg bw/d (10000 ppm) Fertility: 500 mg/kg bw/d	■■■■■ 1987
Daminozide	Rat	Two-generation reproduction, OECD 416, 0, 60, 360 and 1200 mg/kg bw/day	NOEL	Parental: 360 mg/kg bw/d clinical signs and increased water consumption Developmental: 1200 mg/kg bw/d Fertility: 1200 mg/kg bw/d	■■■■■, 1987
Daminozide	Rat	Developmental (gavage), OECD 414, 0, 150, 750 and 1500 mg/kg bw/day	NOEL	Maternal: 150 mg/kg bw/d body weight gain, food consumption Developmental: 1500 mg/kg bw/d Teratogenicity: 1500 mg/kg bw/d	■■■■■ 1993

Daminozide ¹	Rat	Developmental (in diet), 0, 300, 600 and 1000 mg/kg bw/day	NOEL	Maternal: 1000 mg/kg bw/d Developmental: 1000 mg/kg bw/d Teratogenicity: 1000 mg/kg bw/d	██████████ 1979
Daminozide	Rabbit	Developmental (gavage), OECD 414 0, 50 150 and 300 mg/kg bw/day	NOEL	Maternal: 300 mg/kg bw/d Developmental: 300 mg/kg bw/d Teratogenicity: 300 mg/kg bw/d	██████████, 1985
Daminozide	Rabbit	Developmental (gavage), OECD 414, 0, 300, 500 and 700 mg/kg bw/day	NOEL	Maternal: 250 mg/kg bw/d clinical signs and mortality Developmental: 500 mg/kg bw/d slight reduction in ossification and litter weight. Teratogenicity: 1000 mg/kg bw/d	██████████ 2006b

¹ Study considered as supplementary only.

Endpoints in bold have been considered in the risk assessment

According to EFSA Guidance Document (2009), the lowest relevant rodent-specific endpoint from a 2-generation rat study and developmental study should be used in the long-term screening assessment. For daminozide, it is a parental NOEL of 50 mg/kg bw/d (1000 ppm) based on changes in food consumption and body weight from the 2-generation rat study by ██████████ (1987).

Ecotoxicologically relevant endpoint for wild mammals:

Notifier's proposal: The Notifier suggested to use a developmental NOEL of 1200 mg a.s./kg bw/d from the 2-generation rat study by ██████████ (1987) providing the following justification:

Note that the acute and chronic toxicity endpoints reported in the Review Report (2005) for mammals are based on the highest test doses in the acute oral and reproductive study; where both studies reported no major adverse findings. In addition, a second 2-generation rat reproduction study (██████████, 1987) according to OECD 416 reported a NOAEL for development effects and effect on fertility of 1200 mg a.s./kg bw/d. Based on the accumulative findings from the mammalian reproduction studies, as no major effects on reproductive performance were observed at the highest test doses in each study, it is considered scientifically justifiable to use the overall highest NOEL from both studies (i.e. NOEL = 1200 mg a.s./kg bw/d) in the following long-term risk assessment for mammals potentially exposed to daminozide.

Some may consider that ≥1000 mg/kg bw/day is an extreme dose level where, if there is no relevant toxicity seen on repeat dosing, effectively the material may be considered non-toxic, and no risk assessment is needed. Not least for daminozide, because increasing the dose beyond a certain level (here this appears to be lower than 1000 mg/kg

bw/day for daminozide) is unlikely to result in any higher relevant systemic exposure. This is because the absorption of daminozide appears to become saturated beyond a certain dose (██████ 1999, see DAR Addendum, June 2002).

NB mortality, hypoxia, convulsion/tremors seen in ██████ 2006b are considered ultimately a result of lung damage after intubation error or poor intubation and not an intrinsic property of the test material.




Summary of mammalian toxicity studies for daminozide – with comments from Notifier

<i>Study</i>	<i>NOEL/NOAEL (mg/kg bw/day)</i>	<i>LOEL/LOAEL (mg/kg bw/day)</i>	<i>Effects at LOAEL</i>	<i>Reference</i>	<i>Notifier's comment:</i>
<i>13 week oral (gavage) study in the rat. 40, 200 and 1000 mg/kg bw/day</i>	<i>1000 mg/kg bw/day</i>	<i>>1000</i>	<i>No adverse effects were observed in this study</i>	<i>██████ 2005</i>	<i>The substance displayed no toxicity in this study, up to the very extreme limit dose. Increasing the dose further is unlikely to result in any higher relevant systemic exposure, because absorption appears to become saturated at higher doses.</i>
<i>1 year dog dietary 300, 3000, 7500 ppm. A 90 day dog study was not available.</i>	<i>80.5 (3000 ppm)</i>	<i>199 (7500 ppm)</i>	<i>Decreased body weight, acute haemorrhagic enteritis.</i>	<i>██████ 1988a</i>	<p><i>It is noted that a 90 day dog study was not available.</i></p> <p><i>The decrease in body weight gain was less than 6% and the acute haemorrhagic enteritis occurred only in one animal hence is of dubious relevance.</i></p> <p><i>Given the likelihood of saturation of absorption increasing the dose it is unlikely to result in any greater toxicological effect.</i></p> <p><i>The NOAEL relevant for ecotoxicological risk assessment in this study may be considered to be in excess of 199 mg/kg bw/day. It is not the defining study for short term toxicity, because there were other short term toxicity studies testing to higher doses.</i></p>

Study	NOEL/NOAEL (mg/kg bw/day)	LOEL/LOAEL (mg/kg bw/day)	Effects at LOAEL	Reference	Notifier's comment:
<i>Multigeneration (dietary) study in rats at 0, 100, 1000 and 10000 ppm</i>	<i>Parental: 50 (1000 ppm) Developmental: 500 (10000 ppm) Fertility: 500</i>	<i>Parental: 500 Developmental: >500 Fertility: > 500</i>	<i>Parental: changes in food consumption and body weight Developmental: no adverse effect Fertility: no adverse effect</i>	<i>██████████, 1987</i>	<i>Although a parental NOEL has been set based on body weight effects, the magnitudes were not sufficient to be considered adverse (<10% in males, and virtually non-discernible in females), and may be related to the palatability of the diet (food consumption may have been reduced, although this is also hard to discern), hence were not a result of systemic toxicity. Similar effects were not seen in the study below. The NOAEL may be considered to be at least the top dose in this study for ecotoxicological risk assessment purposes. It is not the defining study in this area, because there were similar studies testing to higher doses. In addition, increasing the dose further is unlikely to result in any higher relevant systemic exposure, because absorption appears to become saturated at higher doses.</i>

<i>Study</i>	<i>NOEL/NOAEL (mg/kg bw/day)</i>	<i>LOEL/LOAEL (mg/kg bw/day)</i>	<i>Effects at LOAEL</i>	<i>Reference</i>	<i>Notifier's comment:</i>
<i>Multigeneration (gavage) study in rats at 0, 60, 360 and 1200 mg/kg bw/day</i>	<i>Parental: 360 Developmental: 1200 Fertility: 1200</i>	<i>Parental: 1200 Developmental: >1200 Fertility: > 1200</i>	<i>Parental: clinical signs and increased water consumption Developmental: no adverse effect Fertility: no adverse effect</i>	<i>██████ ██████, 1987</i>	<i>At 1200 mg/kg bw/day there were slight signs of toxicity, characterised by loose faeces, perianal fur staining and post dose salivation. There were, however, no consistent effects of treatment on the bodyweights, food consumption or mating parameters. Similar effects were not seen in the study above. Given the sheer magnitude of dose tested (considerably in excess of the limit dose) the relevance of these signs to any risk assessment is highly dubious, hence the parental NOAEL for ecotoxicological risk assessment purposes may be considered to be at least 1200 mg/kg bw/day.</i>

<i>Study</i>	<i>NOEL/NOAEL (mg/kg bw/day)</i>	<i>LOEL/LOAEL (mg/kg bw/day)</i>	<i>Effects at LOAEL</i>	<i>Reference</i>	<i>Notifier's comment:</i>
<i>Developmental toxicity in rats at 0, 150, 750 and 1500 mg/kg bw/day</i>	<i>Maternal: 150 Developmental: 1500 Teratogenicity: 1500</i>	<i>Maternal: 750 Developmental: ≥ 1500 Teratogenicity: ≥ 1500</i>	<i>Maternal: body weight gain, food consumption Developmental: no adverse effect Teratogenicity: no adverse effect</i>	<i>██████ 1993</i>	<i>The maternal NOAEL has been set in this <u>gavage</u> study at 150 mg/kg based on reduced bodyweight gain coupled with reduced food consumption. This is considered an artefact of the bolus gavage dosing however (the effects were not observed in the ██████ ██████ 1987 dietary study above to any relevant extent) and is therefore not relevant for ecotoxicological risk assessment. Similar, maternal effects were also not observed in ██████, 1979 below. ██████ 1993 does not supersede ██████ ██████ 1987 as the defining study for parental toxicity in the ecotoxicological risk assessment.</i>
<i>Developmental toxicity in rats at 0, 300, 600 and 1000 mg/kg bw/day</i>	<i>Maternal: 1000 Developmental: 1000 Teratogenicity: 1000</i>	<i>Maternal: ≥ 1000 Developmental: ≥ 1000 Teratogenicity: ≥ 1000</i>	<i>Maternal: no adverse effect Developmental: no adverse effect Teratogenicity: no adverse effect</i>	<i>██████, 1979</i>	<i>No effects observed up to the limit dose. It is not the defining study for ecotoxicological risk assessment, because there were similar studies testing to higher doses. In addition, increasing the dose further is unlikely to result in any higher relevant systemic exposure, because absorption appears to become saturated at higher doses.</i>

Study	NOEL/NOAEL (mg/kg bw/day)	LOEL/LOAEL (mg/kg bw/day)	Effects at LOAEL	Reference	Notifier's comment:
<i>Developmental toxicity in rabbits at 0, 50 150 and 300 mg/kg bw/day</i>	<i>Maternal: 300 Developmental: 300 Teratogenicity: 300</i>	<i>Maternal: ≥ 300 Developmental: ≥ 300 Teratogenicity: ≥ 300</i>	<i>Maternal: no adverse effect Developmental: no adverse effect Teratogenicity: no adverse effect</i>	 1985	<i>No effects observed up to the highest dose tested in this study. It is not the defining study in this area, because there were similar studies testing to higher doses.</i>
<i>Developmental toxicity range-finder in rabbits at 0, 300, 500, 700 and 1000 mg/kg bw/day</i>	<i>Not set. Range finding study only</i>	<i>Not set. Range finding study only</i>	<i>-</i>	 2006a	<i>1000 mg/kg/day was considered a maximum tolerated dose in dams. This dose was not lethal but produced scant, soft or liquid faeces and reductions in body weight /feed consumption of approximately 50%. No treatment related developmental toxicity was observed.</i>
<i>Developmental toxicity in rabbits at 0, 250, 500 and 1000 mg/kg bw/day</i>	<i>Maternal: 250 Developmental: 500 Teratogenicity: 1000</i>	<i>Maternal: 500 Developmental: <u>1000</u> Teratogenicity: ≥ 1000</i>	<i>Maternal: clinical signs and mortality Developmental: slight reduction in ossification and litter weight.</i>	 2006b	<i>Administration of the test material at 500 and 1000 mg/kg/day resulted in the death of 7 and 8 animals and the early sacrifice of 2 and 6 animals, respectively. In study summary it was considered that each of these deaths (with the exception of one death in each of these groups that was considered to be the result of intubation accidents) was test material related because they were preceded by adverse clinical observations and/or reductions in body weight gain and feed consumption. In addition, two animals in the 1000 mg/kg/day group aborted and were sacrificed. These</i>

<i>Study</i>	<i>NOEL/NOAEL (mg/kg bw/day)</i>	<i>LOEL/LOAEL (mg/kg bw/day)</i>	<i>Effects at LOAEL</i>	<i>Reference</i>	<i>Notifier's comment:</i>
					<p>abortions were also considered in the study summary to be test material related.</p> <p>In both of these groups, the number of animals with hyperactivity, perinasal substance, hyperpnoea, <u>convulsions (clonic or tonic extension)</u>, tremors, red perioral substance, impaired righting reflex, gasping, ungroomed coat and no faeces in the cage pan were increased or significantly increased. In the 1000 mg/kg/day group, the number of animals with scant faeces, mucoid faeces, decreased motor activity, dehydration, <u>dyspnoea</u>, <u>ptosis</u>, <u>blue or light blue colouring around the mouth and cold to touch</u> (indicating tissue hypoxia) was significantly increased.</p> <p>Some of these effects (<u>those underlined</u>) contrast very strongly with their virtual absence at the same doses in the rabbit range finding study (██████████ 2006a), and gavage studies in the rat (acute, acute neurotox, repeat dose neurotox and developmental, some of which went to 2000 mg/kg or higher), in particular the complete absence of any <u>convulsions and tremors</u>, or <u>hypoxia</u>.</p>

<i>Study</i>	<i>NOEL/NOAEL (mg/kg bw/day)</i>	<i>LOEL/LOAEL (mg/kg bw/day)</i>	<i>Effects at LOAEL</i>	<i>Reference</i>	<i>Notifier's comment:</i>
					<p><i>Daminozide does not cause methaemoglobinaemia or neurotoxicity, and these unusual signs raise the question of whether the right test substance was administered, or whether the real cause of death in these animals was <u>lung damage</u> caused by <u>intubation error</u> (the material does affect mucous membrane – see the eye irritation study). Convulsions and tremors are also often associated with hypoxia in the brain.</i></p> <p><i>Very importantly the <u>convulsions and tremors</u> and <u>hypoxia</u> only occurred in animals that ultimately died. In survivors these particular signs were absent. (scant or soft faeces were still apparent however).</i></p> <p><i>Given that it is unlikely that the wrong test substance was administered (there was commonality of soft or scant faeces with other studies), the origin of the <u>convulsions and tremors</u>, <u>hypoxia</u> leading to death are more likely to be due to test material entering and damaging the lungs.</i></p> <p><i>Given the nature of the signs and mortality and the incidence observed at 500 mg/kg bw/day, it would not be unreasonable to have expected total group loss at</i></p>

<i>Study</i>	<i>NOEL/NOAEL (mg/kg bw/day)</i>	<i>LOEL/LOAEL (mg/kg bw/day)</i>	<i>Effects at LOAEL</i>	<i>Reference</i>	<i>Notifier's comment:</i>
					<p>1000 mg/kg bw/day. The incidence was similar to the 500 mg/kg bw/day group however and again this more likely indicates test intubation issues.</p> <p>Overall there were no intrinsically treatment related maternal effects in this study relevant to the ecotoxicological risk assessment and the maternal NOAEL from this study for this purpose is >1000 mg/kg bw/day.</p> <p>With respect to the abortions seen, these can be common in rabbits and not necessarily treatment related. The occurrence in this study does not increase above what might be considered incidental, and a high background incidence was seen in the rabbit study with "Alar" (██████████ 1985).</p> <p>The number of foetuses with alterations was significantly increased in the 1000 mg/kg/day group. This increase included a significant increase in the number of foetuses with thickened ribs. The average number of ossified forelimb phalanges was significantly reduced in this group. No other gross external, soft tissue or skeletal foetal alterations</p>

<i>Study</i>	<i>NOEL/NOAEL (mg/kg bw/day)</i>	<i>LOEL/LOAEL (mg/kg bw/day)</i>	<i>Effects at LOAEL</i>	<i>Reference</i>	<i>Notifier's comment:</i>
					<p><i>(malformations or variations) or differences in ossification sites per litter were caused by the test material.</i></p> <p><i>These effects however are known to recover rapidly before or immediately after birth and are not relevant for the ecotoxicological risk assessment. The developmental NOAEL was set accordingly at the highest dose tested.</i></p>

RMS comment:

More details and comments on four studies which are the most crucial for setting of the long-term ecotoxicologically relevant endpoint for wild mammals are provided below.

1) Two-generation [REDACTED] 1987:

Parental NOEL: 50 mg/kg bw/d (1000 ppm)

Body weights:

F1: Body weights for the 500 mg/kg bw/d (10000 ppm) males were significantly different from those of controls at weeks 11 and 12, and 15 through 19. However, the effect levels were 3.2 - 8.7% compared to control. In the old DAR (1999), it is stated that these effects have dose-response character. It is not clear since the body weights in the 50 mg/kg bw/d (1000 ppm) are higher than in the 5 mg/kg bw/d (100 ppm). Therefore, these differences in body weights are not considered biologically significant by RMS.

Food consumption:

F0 and F1: Food consumption for females was significantly higher than that of controls during Weeks 2 through 7 for the 500 mg/kg bw/d (10000 ppm) group which is not considered as an adverse effect.

→ **Ecotoxicologically relevant parental NOAEL: 500 mg/kg bw/d (10000 ppm)**, i.e. RMS agrees with the Notifier's comment on this study (see the table above).

2) Two-generation [REDACTED] 1987:

Parental NOEL: 360 mg/kg bw/d

Water consumption:

F0: Increased water consumption was observed in males in 1200 mg/kg/d group (day 86-91). In the lower dose groups it was similar to control.

Clinical observations:

F0: in 1200 mg/kg/d group animals with loose faeces, unusual odour – from week 4 of treatment. All animals perianal fur staining from week 10 and excess salivation from week 11. Similar observation also in F1.

The observed effects are considered as signs of the slight toxicity that are not likely to have effects on reproduction in nature. No consistent effects of treatment on the bodyweights, food consumption or mating parameters.

→ **Ecotoxicologically relevant parental NOAEL: 1200 mg/kg bw/d**, i.e. RMS agrees with the Notifier's comment on this study (see the table above).

3) Developmental rat (gavage) [REDACTED] 1993:

Maternal NOEL: 150 mg/kg bw/d

Dose-related decrease of body weight gain and food consumption in 750 and 1500 mg/kg/d groups. However, the maternal toxicity did not lead to any developmental or teratogenicity effects.

→ **Maternal NOEL is not considered ecotoxicologically relevant**, i.e. RMS agrees with the Notifier's comment on this study (see the table above).

4) Developmental rabbit (gavage) [REDACTED] 2006b:

Maternal NOEL: 250 mg/kg bw/d

Adverse clinical observations and mortality in 500 and 1000 mg/kg/d groups. The maternal toxicity in 500 and 1000 mg/kg/d group did not lead to any developmental or teratogenicity effects.

Developmental NOEL: 500 mg/kg bw/d

In 1000 mg/kg/d group, a 10% reduction in foetal weights (15% in males, 8% in females), an increase in the overall incidence of foetal alterations (foetal basis, but not litter basis) and an interrelated (to the reduction in foetal weight) reduction in the ossification of forelimb phalanges occurred. The increased number of foetal alterations included a significant increase in the number of foetuses with thickened ribs, a common variation.

→ **Ecotoxicologically relevant developmental NOEL: 500 mg/kg bw/d**, RMS disagrees with the Notifier's comment on this study (see the table above).

RMS is of opinion that the adverse effects on foetuses in 1000 mg/kg/d (reduction of foetal weights, increase incidence of foetal alterations) in connection with maternal toxicity (adverse clinical observations and 33% mortality) should be considered as ecotoxicologically relevant. Moreover, two abortions (out of 24) were reported at this dose and considered to be test material related by the study author while none abortion was reported in control or lower doses.

According to the Notifier, increased maternal mortality and adverse clinical signs were likely due to the test material entering and damaging the lungs caused by intubation error (the material does affect mucous membrane – see the eye irritation study; convulsions and tremors are also often associated with hypoxia in the brain).

However, since the test substance related effects cannot not be excluded RMS insists on ecotoxicologically relevant developmental NOEL of 500 mg/kg bw/d derived from this study.

Two other studies relevant for the wild mammal risk assessment which were available (developmental toxicity in rat – [REDACTED], 1979 and developmental toxicity in rabbit – [REDACTED], 1985) indicated no adverse effects up to the highest concentration tested.

Based on the data provided above, RMS proposes **NOAEL of 500 mg/kg bw/d as the long-term ecotoxicologically relevant endpoint for wild mammals** derived from the developmental rabbit study by [REDACTED] (2006b).

It is noted that no such adverse developmental effects observed in [REDACTED] (2006b) were noted in the other studies, however, such high doses (≥ 1000 mg/kg/ bw/d) were only tested in developmental studies on rat ([REDACTED] 1993 and [REDACTED] 1979). No other developmental study on rabbit is available, apart from the pilot study by [REDACTED] (2006a) with the highest dose tested of 300 mg/kg/ bw/d.

The selection of ecotoxicologically relevant endpoint to be used in the reproductive risk assessment for wild mammals should be discussed in peer review.

B.9.2 Risk assessment for birds and other terrestrial vertebrates

An ecological risk assessment in relation to the risk to birds has been undertaken in accordance with the 'Guidance of EFSA Risk Assessment for Birds and Mammals', EFSA Journal 2009 7(12):1438.

Intended application pattern relevant to the uses of daminozide are given in the table below.

Table B.9.2-1 Intended application pattern

Crop	Timing of application BBCH	Method of application	Number of applications	Interval between applications (min.)	Maximum application rate, individual treatment	
					Product [kg/ha]	Daminozide [kg a.s./ha]
Ornamentals (Protected)	<50	Over spray (Gantry)	1 - 5	7 days	9	7.65
Ornamentals (Field)	<50	Foliar*	1 - 5	7 days	5	4.25

The risk assessment was performed for field use (4.25 kg a.s./ha). For protected use (7.65 kg a.s./ha), the risk assessment for birds and mammals should be performed assuming the same exposure as for a field use, unless it is indicated that the uses will be restricted to permanent greenhouses (based on Technical report on the outcome of the pesticides peer review meeting on general recurring issues in ecotoxicology (EFSA, 2015)). This was not indicated in the GAP. Therefore, the risk is considered low for permanent greenhouses and no risk assessment is required for birds and mammals, however, for protected use other than permanent greenhouses, the risk assessment for birds and mammals assuming the same exposure as for a field use was carried out.

B.9.2.1 Risk assessment for birds

Exposure of birds will be predominantly dietary, through the consumption of residues on food items. Direct exposure of birds to applications is considered unlikely, since at the time of application and for a short period thereafter, most birds will leave the immediate vicinity of spray operations in response to the human disturbance.

According to the EFSA guidance document the risk assessment follows a screening step, then if needed a first tier assessment followed by a higher tier risk assessment if required.

Screening assessment

Daily dietary dose (DDD) for single and multiple applications:

For acute risk assessment - use the shortcut value and the application rate in kg/ha; for multiple applications multiply the single application DDD by an appropriate Multiple Application Factor for 90th percentile residue data (MAF₉₀):

$$\text{DDD}_{\text{single application}} = \text{application rate (kg/ha)} * \text{shortcut value}$$

$$\text{DDD}_{\text{multiple application}} = \text{DDD}_{\text{single application}} * \text{MAF}_{90}$$

For long-term risk assessment - use the shortcut value, mean residue MAF_m , appropriate STE or LTE TWA, and calculate the DDD for single or multiple applications as appropriate:

$$DDD = \text{Application rate} \times \text{Shortcut value} \times \text{TWA} \times MAF_m$$

If the toxic effect is considered to be caused by long term effects a time weighted average factor of 0.53 should be used.

Calculation of Toxicity Exposure Ratio (TER)

The equation for calculation of TER is given below:

$$TER = \frac{\text{Toxicity value}}{DDD}$$

The calculation of the TER values is presented in the table below.

Table B.9.2.1-1 Avian screening assessment for the proposed use of Dazide Enhance on ornamentals

Table B.7.2.1-1 Avian screening assessment for the proposed use of Diazide Enhance on ornamentals								
Crop	Indicator spp.	Time scale & shortcut value	MAF	TWA	DDD (mg/kg bw)	Endpoint (mg/kg bw)	TER	Trigger value
Field use (application rate: 5 x 4.25 kg a.s./ha with 7 day interval)								
Ornamentals	Small insectivorous bird	Acute: 46.8	1.9	-	378	4248 ^a	11.2	10
		Long-term: 18.2	2.4	0.53	98.4	79.9	0.81	5
Protected use (application rate: 5 x 7.65 kg a.s./ha with 7 day interval)								
Ornamentals	Small insectivorous bird	Acute: 46.8	1.9	-	680	4248 ^a	6.2	10
		Long-term: 18.2	2.4	0.53	177	79.9	0.45	5

^a Extrapolated from the reported endpoint of >2250 mg a.s./kg bw, based on a factor of 1.888

MAF: Multiple application factor; TWA: time weighted average; DDD: daily dietary dose

Value(s) in bold are below the trigger value

For field use, the acute TER value is above the trigger value of 10, indicating a low acute risk, while the long-term TER value is below the trigger value of 5. For protected use (other than permanent greenhouses), the both acute and long-term TER values are below the relevant triggers. Therefore, Tier I assessment is required.

Tier I assessment

A Tier I long-term risk assessment has been conducted and the TER values for the generic focal species foraging in ornamentals are presented in the table below.

Table B.9.2.1-2 Tier I TER values for birds foraging in treated ornamentals

Generic focal species	Scenario	Time scale & shortcut value	MAF	TWA	DDD (mg/kg bw)	Endpoint (mg/kg bw/d)	TER _{LT}	Trigger value
Field use (application rate: 5 x 4.25 kg a.s./ha with 7 day interval)								
Small insectivorous bird "tit"	Application to plant	Long-term: 18.2	2.4	0.53	98.4	79.9	0.81	5
Small insectivorous / worm feeding bird "thrush"	Application to plant – exposure to underlying ground	Long-term: 2.7	2.4	0.53	14.6	79.9	5.46	5
Protected use (application rate: 5 x 7.65 kg a.s./ha with 7 day interval)								
Small insectivorous bird "tit"	Application to plant	Acute: 46.8	1.9	-	680	4248 ^a	6.2	10
		Long-term: 18.2	2.4	0.53	177	79.9	0.45	5
Small insectivorous / worm feeding bird "thrush"	Application to plant – exposure to underlying ground	Acute: 7.4	1.9	-	108	4248 ^a	39.33	10
		Long-term: 2.7	2.4	0.53	14.6	79.9	26.27	5

MAF: Multiple application factor; TWA: time weighted average; DDD: daily dietary dose

Value(s) in bold are below the trigger value

The Tier I long-term TER values demonstrate a low risk to birds foraging on ground dwelling insects ("thrush") but not for birds feeding on foliar insects ("tit"). A refined risk assessment for small insectivorous birds, "blue tit" as the representative species, has therefore been conducted.

Refined long-term dietary risk assessment

The Notifier provided the refined long-term dietary risk assessment which is presented below.

Notifier's proposal:

"The scenario of concern is a small insectivorous bird feeding on contaminated foliar dwelling insects. The blue tit (Parus caeruleus) is identified in EFSA's Bird and Mammal Guidance Document (2009) as the representative small insectivorous bird foraging in ornamentals that are treated with canopy directed applications.

The first tier risk assessment assumes as a worst-case that 100% of the bird's diet will consist of contaminated food items from the treated area. As with any area of crop production, nurseries growing high quality ornamentals intended for sale are likely to maintain pest populations to low levels in order to prevent reduction in commercial value of the products. For example, employing integrated systems following good plant protection practice for optimal practice in protecting crops against their overall pest spectrum (e.g. see EPPO Standards, series PP2 Good Plant Protection Practice (GPP)¹). Therefore, there will be limited invertebrates present on the foliage of

¹ <http://archives.eppo.int/EPPOStandards/gpp.htm>

the plants and it will not be possible for a small insectivorous bird to obtain its dietary needs from this food source over a long-term scenario.

Furthermore, as the crop will be intensively managed, disturbance of birds will be high. Overall, ornamental nurseries are not considered to provide attractive foraging areas for birds, especially those foraging for insects from the foliage of plants. Thus, the long-term exposure to birds from the proposed use of Dazide Enhance is considered to be negligible and consequently the risk is acceptable.

However, in demonstrating an acceptable risk via a TER_{LT} greater than the trigger value, the following input parameters were reviewed:

A) Composition of diet obtained from treated area (PD)

*During March to September, the diet of a blue tit may be assumed to consist entirely of foliage arthropods (PD = 1). Outside this period, nuts and seeds from trees enter the diet but probably never make up more than 50% (Aagaard, 2014²). Therefore, the **PD of 1** is not adjusted for this scenario.*

² Aagaard, A. (2014) Pesticide risk assessment for birds and mammals. Selection of relevant species and development of standard scenarios for higher tier risk assessment in the Northern Zone in accordance with Regulation EC 1107/2009. Version 1.1

B) Proportion of an animal's daily diet obtained in habitat treated with pesticide (PT)

Blue tits are fairly common in rural gardens, deciduous hedgerows and parks. The habitat preferences also include orchards and nurseries, providing there are suitable nest-holes available.

Ornamentals and nursery cultures are very variable; from small plants in nurseries to large plants grown under conditions reminiscent of those in orchards (Aagaard 2014). As data for ornamentals is not available, data for orchards is considered as a worst-case since orchards contain a wide abundance of arthropods. A study of orchards in the UK is considered for refining the PT. Twenty blue tits were radio-tracked to estimate the active time spent in orchards (Prosser 2010³, Finch & Payne 2006⁴). The percentage of active time spent by radio-tagged blue tits was divided by the total sample of tracked birds ("all birds") as well as for the subsample of birds that actually used the orchard ("consumers only") during April to September. The findings are summarised in the following table:

Percentage of active time spent by radio-tagged blue tits in orchards in the UK, presented as 90th percentile of the modelled PT distribution

<i>Crop</i>	<i>Period</i>	<i>No. of birds</i>	<i>Mean PT</i>	<i>90th PT</i>	<i>95th PT</i>	<i>Reference</i>
<i>All birds:</i>						
<i>Orchard</i>	<i>Apr – Sep</i>	<i>20</i>	<i>0.21</i>	<i>0.55</i>	<i>0.67</i>	<i>Finch & Payne 2006</i>
		<i>20</i>	<i>-</i>	<i>0.53</i>	<i>-</i>	<i>Prosser 2010</i>
<i>Consumers only:</i>						
<i>Orchard</i>	<i>Apr – Sep</i>	<i>16</i>	<i>0.27</i>	<i>0.58</i>	<i>0.68</i>	<i>Finch & Payne 2006</i>
		<i>16</i>	<i>-</i>	<i>0.57</i>	<i>-</i>	<i>Prosser 2010</i>

*The PT can therefore be adjusted to represent a more realistic portion of the bird's diet that is actually obtained from the treated crop. A **PT of 0.53** is considered to satisfy the 90th percentile of birds that both visit and are "consuming" in the crop as recommended in the report by Aagaard (2014^{Chyba! Zázložka není definována.}) for orchards. A PT for "all" birds is considered since the tracked birds were caught inside the orchard or along the orchard edge.*

C) Residue unit dose (RUD)

*The long-term risk assessment for insectivorous birds can be refined using a **RUD of 5.1 mg/kg** for foliar insects. This refinement is supported by the data presented in Fischer and Bowers (1997), as given in Appendix II, Table*

³ Prosser, P. 2010. Consolidation of bird and mammal PT data for use in risk assessment. Food and Environment Research Agency, UK.

⁴ Finch, E. & Payne, M. (2006). Bird and mammal risk assessment: refining the proportion of diet obtained in the treated crop area (PT) through the use of radio tracking data. Advisory Committee on Pesticides, Environmental Panel, SC 11449.

4 of SANCO/4145/2000, where insect / invertebrate residue data compiled from 24 field applications were analysed for mean residue levels following both foliar and soil- incorporation.

Refined TER_{LT} for small insectivorous bird, Blue tit

The refined long-term TER for the blue tit is presented in the table below, with consideration of the refinements discussed above.

Refined chronic TER value for small insectivorous birds (blue tit) foraging in treated ornamentals (application rate: 5 x 4.25 kg a.s./ha with 7 day interval)

Representative species	FIR /bw^a	Refined RUD^b	PD	PT	MAF	f_{TWA}	DF	DDD (mg/kg bw)	End-point (mg/kg bw/d)	TER_{LT}	Trigger value
Small insectivorous bird "blue tit"	0.86	5.1	1	0.53	2.4	0.53	1	12.6	79.7	6.34	5

^a FIR/bw: food intake rate per body weight according to EFSA (2009)

^b RUD: residues per unit dose based on the data presented by Fischer and Bowers (1997)

DF: deposition factor

MAF: multiple application factor

TWA: time weighted average factor

PT: proportion of diet obtained in the treated area

PD: proportion of food type in the diet

DDD: daily dietary dose

Based on the refined assessment with consideration to portion of diet obtained from the treated area (PT), and a refined RUD for foliar insects, the TER_{LT} for blue tits is above the trigger value. Hence the long-term risk to birds is acceptable.

The long-term TERs are considered to be conservative as it should be highlighted again that the toxicity endpoint used in the risk assessment is based on the highest test dose from the bobwhite quail reproductive study. In the avian reproductive study there were no treatment-related mortalities, overt signs of toxicity or treatment-related effects upon body weight or feed consumption at any of the concentrations tested. Additionally, there were no treatment-related effects upon any of the reproductive parameters measured. The NOEC for the tested birds exposed to daminozide in the diet during the study was 1000 ppm a.s., the highest concentration tested. Hence the "true" NOEC is indeed higher, demonstrating that daminozide is non-toxic to birds even from chronic exposure."

RMS comments on Notifier's proposal of refined long-term risk assessment:

RMS agrees to use the blue tit (*Cyanistes caeruleus*) as a specific focal species. It is identified in EFSA GD (2009) as the representative species for ornamentals with canopy directed application and it is a widespread and common

species throughout the Europe. Its primary habitat is deciduous woodland but it also occurs in coppice, overgrown marshes and mires etc. The species is frequent in parks, gardens and other man-made habitats (Aagaard, 2014). In addition, the blue tit is considered sufficiently protective also for other species due to its low body weight.

Ad A): RMS agrees to use PD of 1.

Ad B): RMS agrees to use the data for orchards since data for ornamentals are not available. However, RMS considers more relevant to use the “consumer” approach, which is the most conservative PT. It is agreed to use the 90th percentile PT. Thus, the PT value proposed by RMS is **0.58**.

Ad C): RMS disagrees with using of a RUD of 5.1 mg/kg for foliar insects in the long-term risk assessment for insectivorous birds. In the current EFSA Guidance Document (EFSA 2009), the food categories and RUD values originally used in SANCO/4145/2000 were revised, based on new or updated extensive databases. Therefore, it is not justified to use out-dated RUD values from SANCO/4145/2000. Further it is noted that the RUD value relevant for blue tit (mean RUD value of **21.0** for foliar dwelling insects) is already incorporated in the Tier I long-term shortcut value of 18.2.

The TER calculation using PT and RUD values proposed by RMS is presented below.

Refined long-term risk assessment: TER calculation

$$\text{DDD (mg/kg bw/d)} = (\text{FIR} / \text{bw}) * \text{RUD} * \text{PT} * \text{PD} * \text{MAF} * f_{\text{TWA}} * \text{AR}$$

Table B.9.2.1-3 Refined TER value for small insectivorous birds (blue tit) foraging in treated ornamentals

Representative species	FIR / bw ^a	Mean RUD foliar insect ^b	PD	PT	MAF	f _{TWA}	AR (kg a.s./ha)	DDD (mg/kg bw)	End-point (mg/kg bw/d)	TER _{LT}	Trigger value
Field use (application rate: 5 x 4.25 kg a.s./ha with 7 day interval)											
Small insectivorous bird “blue tit”	0.86	Long-term: 21.0	1	0.58	2.4	0.53	4.25	56.6	79.7	1.41	5
Protected use (application rate: 5 x 7.65 kg a.s./ha with 7 day interval)											
Small insectivorous bird “blue tit”	0.86	Acute: 54.1	1	0.58	2.4	-	7.65	495.4	4248	8.57	10
	0.86	Long-term: 21.0	1	0.58	2.4	0.53	7.65	101.9	79.7	0.78	5

^a FIR/bw: food intake rate per body weight according to EFSA (2009)

^b RUD: residues per unit dose according to EFSA (2009)

DF: deposition factor

MAF: multiple application factor

f_{TWA}: time weighted average factor

PT: proportion of diet obtained in the treated area

PD: proportion of food type in the diet

AR: single application rate

DDD: daily dietary dose

All the TER values remained below the relevant triggers. No further refinement was available. Therefore, the high dietary long-term risk for field use and the high dietary acute and long-term risk for protected use (other than permanent greenhouses) has been concluded for small insectivorous bird (blue tit).

Dietary risk to birds from metabolites

No studies on residues in plants were available.

As methanol was identified as a potentially relevant metabolite in surface water and soil compartments, potential exposure of birds to this metabolite should be assessed. No toxicity data were available for the metabolite methanol.

However, based on the physical-chemical properties of methanol; i.e. high vapour pressure (1.69×10^4 Pa at 25°C) from soil, it is assumed that methanol will be present in relevant food items in rather small amounts. Therefore, the exposure of birds to methanol is expected to be negligible and the risk is considered to be covered with the risk assessment for the parent daminozide. No special dietary risk assessment for methanol is required.

Exposure to birds will be predominately dietary, through the consumption of residue on food items e.g. sprayed crop, weeds and insects. There is also a possibility that birds can be exposed via drinking water, see section below on exposure via drinking water.

Risk assessment for drinking water exposures

The risk assessment for drinking water exposures follows the guidance of the EFSA Journal 2009 7(12):1438 Section 5.5.

The guidance document states that the leaf scenario is needed for leafy vegetables forming heads and for other leafy vegetables (e.g. cauliflower) where the morphology facilitates the collection of rain or irrigation water. The uses of the daminozide do not include such crops. Therefore, the leaf scenario is not relevant for this assessment. The relevant scenario for the proposed uses is the puddle scenario, which is relevant for all outdoor uses.

According to the EFSA guidance document an assessment for puddle scenario is not required when the ratio of effective application rate (in g/ha) to the relevant endpoint (in mg/kg bw/d) does not exceed 50 in the case of less sorptive substances ($K_{oc} < 500$ L/kg) or 3000 where the $K_{oc} \geq 500$ L/kg.

Daminozide has a K_{oc} of 26.6 L/kg (mean). The maximum effective rate of use of Dazide Enhance is calculated for field use (5 x 4.25 kg a.s./ha with a 7 day interval) and for protected use other than greenhouses (5 x 7.65 kg a.s./ha with a 7 day interval) for ornamentals. The effective application rate is calculated by multiplying the proposed application rate by a MAF value based on the DT_{50} in soil (EFSA, 2009) for the active substance; for daminozide the geometric mean soil DT_{50} is 0.12 days.

Methanol has a K_{oc} of 1.0 L/kg. For the calculation of methanol PECs it was assumed that daminozide was instantly degraded, and therefore daminozide application rates were corrected for the relative molecular masses of parent and metabolite, as well as for the maximum formation of methanol. Therefore the maximum effective rate of methanol is calculated for field use (5 x 4.25 kg a.s./ha with a 7 day interval) and for protected use other than

greenhouses (5 x 7.65 kg a.s./ha with a 7 day interval) for ornamentals. The effective application rate is calculated by multiplying the proposed application rate by a MAF value based on the DT₅₀ in soil for the metabolite; for methanol the soil DT₅₀ is 3.9 days (geomean; calculated). Note that the soil DT₅₀ is considered to be conservative since exposure to the metabolite is expected to be limited based on the physical-chemical properties of methanol; i.e. high vapour pressure (1.69 x 10⁴ Pa at 25°C) from soil.

The table below summarises the ratios for daminozide and its metabolite methanol using both the acute and long-term endpoints.

Table B.9.2.1-4 Ratios of effective application rate to endpoints for daminozide and its metabolite

Test substance	Time scale	Application rate (g a.s./ha)	MAF	Effective application rate (g a.s./ha)	Endpoint	Ratio	Trigger value
Daminozide	Field use (application rate: 5 x 4.25 kg a.s./ha with 7 day interval)						
	Acute	4250	1.00 ^a	4250	4248 mg/kg bw ^b	1.00	50
	Long-term				79.7 mg/kg bw/d	53.3	
	Protected use (application rate: 5 x 7.65 kg a.s./ha with 7 day interval)						
	Acute	7650	1.00 ^a	7650	4248 mg/kg bw ^b	1.80	50
	Long-term				79.7 mg/kg bw/d	95.7	
Methanol	Field use (application rate: 5 x 4.25 kg a.s./ha with 7 day interval)						
	Acute	4250	1.40 ^c	5950	424.8 mg/kg bw ^d	14.00	50
	Long-term				7.97 mg/kg bw/d ^e	747	
	Protected use (application rate: 5 x 7.65 kg a.s./ha with 7 day interval)						
	Acute	7650	1.40 ^c	10710	424.8 mg/kg bw ^d	25.21	50
	Long-term				7.97 mg/kg bw/d ^e	1344	

^a Based on the geomean soil DT₅₀ of 0.12 days

^b Extrapolated from the reported endpoint of >2250 mg a.s./kg bw, based on a factor of 1.888

^c Based on the soil DT₅₀ of 3.9 days (geomean)

^d There are no toxicity data available for the metabolites methanol, therefore the amebolite has been assumed to be 10 times more toxic than the parent (LD₅₀ = 4248 mg a.s./kg bw / 10 = 424.8 mg a.s./kg bw).

^e There are no toxicity data available for the metabolite methanol, therefore the amebolite has been assumed to be 10 times more toxic than the parent (NOEL = 79.7 mg a.s./kg bw/d / 10 = 7.97 mg a.s./kg bw/d).

MAF: Multiple application factor

The above acute ratios are below the trigger value of 50 indicating an acceptable risk to birds *via* drinking water contaminated from the proposed use of daminozide. However, the long-term ratios are above the trigger value and a Tier 1 drinking water assessment is required.

The long-term drinking water assessment is presented in the table below. The default drinking water rate (DWR) given in EFSA's Bird and Mammals Guidance (2009) has been used, along with the calculated PEC_{puddle}, and toxicity endpoints to calculate the TER.

Table B.9.2.1-5 Tier I avian drinking water assessment (puddle scenario) for the proposed use of Dazide Enhance

Test substance	Generic spp.	Time-scale	DWR (L/kg bw/d)	PECpuddle (mg a.s./L)	Daily dose (mg a.s./kg bw)	Endpoint (mg a.s./kg bw/d)	TER	Trigger value
Daminozide	Field use (application rate: 5 x 4.25 kg a.s./ha with 7 day interval)							
	Small granivorous bird "linnet"	Long-term	0.46	7.10	3.27	79.7	24.52	5
	Protected use (application rate: 5 x 7.65 kg a.s./ha with 7 day interval)							
	Small granivorous bird "linnet"	Long-term	0.46	12.77	5.87	79.7	13.58	5
Methanol	Field use (application rate: 5 x 4.25 kg a.s./ha with 7 day interval)							
	Small granivorous bird "linnet"	Long-term	0.46	19.77	9.09	7.97	0.88	5
	Protected use (application rate: 5 x 7.65 kg a.s./ha with 7 day interval)							
	Small granivorous bird "linnet"	Long-term	0.46	35.16	16.17	7.97	0.49	5

The above TER values for daminozide are greater than the trigger value of 5, demonstrating low long-term risk to birds exposed to Dazide Enhance *via* drinking water. However, the TER values for metabolite methanol are below the trigger value of 5, indicated high risk *via* drinking water. No further refinement was available.

Risk for Bioaccumulation and Secondary Poisoning

Substances with a high bioaccumulation potential could theoretically bear a risk of secondary poisoning for birds if feeding on contaminated prey like fish or earthworms. For organic chemicals, a log $P_{ow} > 3$ is used to trigger an in-depth evaluation of the potential for bioaccumulation.

As the log P_{ow} of daminozide and methanol are less than the trigger value of 3 (log P_{ow} at pH 7 = -1.5 and -0.77⁵, respectively), the risk to birds from secondary poisoning is considered to be negligible and no further consideration is required.

Conclusion – risk to birds

No acute risks and no reproductive risks from drinking water exposure and secondary poisoning were identified for birds for field use.

No acute and reproductive risks were identified for birds for protected use in permanent greenhouses.

⁵ Material Safety Data Sheet – Methanol (CAS # 67-56-1). <https://fscimage.fishersci.com/msds/14280.htm>

High dietary reproductive risk was concluded for small insectivorous bird (blue tit) for field use.

High dietary acute and reproductive risk was concluded for small insectivorous bird (blue tit) for protected use (other than permanent greenhouses).n added.

High risk from drinking water exposure was identified for methanol.

B.9.2.2 Risk assessment for mammals

The risk assessment procedure for wild mammals follows the same principles as described for birds, i.e. EFSA guidance document.

Screening assessment

The calculation of the TER values is presented in the table below.

Table B.9.2.2-1 Mammal screening assessment for the proposed use of Dazide Enhance on ornamentals

Crop	Indicator spp.	Time scale & shortcut value	MAF	TWA	DDD (mg/kg bw)	Endpoint (mg/kg bw)	TER	Trigger value
Field use (application rate: 5 x 4.25 kg a.s./ha with 7 day interval)								
Ornamentals	Small herbivorous mammal	Acute: 136.4	1.9	-	1101	>5000	>4.54	10
		Long-term: 72.3	2.4	0.53	391	500	1.28	5
Protected use (application rate: 5 x 7.65 kg a.s./ha with 7 day interval)								
Ornamentals	Small herbivorous mammal	Acute: 136.4	1.9	-	1983	>5000	>2.52	10
		Long-term: 72.3	2.4	0.53	704	500	0.71	5

^a Extrapolated from the reported endpoint of >2250 mg a.s./kg bw, based on a factor of 1.888

MAF: Multiple application factor; TWA: time weighted average; DDD: daily dietary dose

Value(s) in bold are below the trigger value

All TER values are below the relevant triggers. Therefore, Tier I assessment is required.

Table B.9.2.2-2 Tier I TER values for mammals foraging in treated ornamentals

Generic focal species	Scenario	Time scale & shortcut value	MAF	TWA	DDD (mg/kg bw)	Endpoint (mg/kg bw/d)	TER _{LT}	Trigger value
Field use (application rate: 5 x 4.25 kg a.s./ha with 7 day interval)								
Small insectivorous mammal “shrew”	Application to plant – exposure to underlying ground	Acute: 5.4	1.9	-	43.62	>5000	>115	10
		Long-term: 1.9	2.4	0.53	10.27	500	48.69	5
Small herbivorous mammal “vole”	BBCH 40-49	Acute: 136.4	1.9	-	1101	>5000	>4.54	10
		Long-term: 72.3	2.4	0.53	391	500	1.28	5
Small omnivorous mammal “mouse”	Application crop directed BBCH 10-49	Acute: 17.2	1.9	-	139	>5000	>35.97	10
		Long-term: 7.8	2.4	0.53	44.85	500	11.15	5
Protected use (application rate: 5 x 7.65 kg a.s./ha with 7 day interval)								
Small insectivorous mammal “shrew”	Application to plant – exposure to underlying ground	Acute: 5.4	1.9	-	78.49	>5000	>63.70	10
		Long-term: 1.9	2.4	0.53	17.52	500	28.54	5
Small herbivorous mammal “vole”	BBCH 40-49	Acute: 136.4	1.9	-	1975	>5000	>2.53	10
		Long-term: 72.3	2.4	0.53	704	500	0.71	5
Small omnivorous mammal “mouse”	Application crop directed BBCH 10-49	Acute: 17.2	1.9	-	250	>5000	>20.00	10
		Long-term: 7.8	2.4	0.53	75.90	500	6.59	5

MAF: Multiple application factor; TWA: time weighted average; DDD: daily dietary dose

Value(s) in bold are below the trigger value

All TER values are above the relevant triggers, except for acute and long-term TER values for small herbivorous mammal "vole". Therefore, further consideration is required.

Refined long-term dietary risk assessment

The Notifier provided the refined long-term dietary risk assessment which is presented below.

The scenario of concern is a small herbivorous mammal feeding solely on contaminated grass, as the crop itself is not an attractive food source. The common vole (Microtus arvalis) is identified in EFSA's Bird and Mammal Guidance Document (2009) as the representative small herbivorous mammal foraging in ornamentals.

According to the diverse good agricultural practice in Europe, many nurseries will be completely free of vegetation between plants; hence ornamentals without ground cover are not considered to be an appropriate habitat for small mammals. Therefore, for these scenarios no refinement for the long-term risk to voles has to be considered. However, as a conservative approach a risk assessment for voles has been conducted for scenarios where some ground vegetation may be present in ornamental nurseries, although these areas are still not suitable environments for voles since they prefer dense vegetation and minimal disturbances. Furthermore, it should be mentioned that

in many regions voles are regarded as pests in nurseries and are actively controlled. Nevertheless, the following refinements are considered in calculating the refined TER values for voles foraging in daminozide treated nurseries.

A) Composition of diet obtained from treated area (PD)

The vole feeds on a broad variety of plants and exhibits a pronounced selective food intake (Rinke, 1990⁶). In grassland areas the diet consists of 35% monocotyledonous (grass) and 65% dicotyledonous plants. The dicotyledonous *Taraxacum officinale* (23.95%) and *Trifolium pratense* (18.6%) are by far the most important food items, accounting for 42.55% of the diet. All other plant species contribute between 0.01 to 5.91% of the diet (Rinke, 1990; Rinke, 1991⁷). Therefore, the diet that would be relevant for this scenario is assumed to consist of 35% monocotyledonous plants and 65% dicotyledonous plants (**PD: 0.35 grass; 0.65 non-grass herbs**). This refinement has been applied only to the long-term risk assessment.

B) Proportion of an animal's daily diet obtained in habitat treated with pesticide (PT)

As a weight-of-evidence for PT the following arguments are presented:

- The optimum habitat of common voles comprises large open, dry, uniform grassy areas (Schröpfer and Hildenhagen, 1984⁸). This is further supported by Delattre et al. (1992)⁹ who reported the importance of permanent grassland in farmland for the population dynamics of common voles. When grass cover diminished so did populations of common voles and an increase in grassland was followed by increases in vole populations.
- Results of a field study in an Integrated Pest Management (IPM) apple orchard in Poland indicated that *Microtus arvalis* was the dominant rodent species with a proportion of 70% to 90% of the rodent population. During all seasons the least number of rodent colonies was found on plots with herbicidal weeding while the highest abundance was observed on those study plots with herbaceous plant cover not cut until autumn. Cutting the plant cover during summer reduced the numbers of voles to a level comparable on plots with herbicidal weeding (Jaworska et al., 1995)¹⁰. This study again demonstrates the dependency of common voles on a permanent and undisturbed vegetation cover.
- The population density of common voles undergoes major fluctuations both within a season and between years (Mackin-Rogalska and Nabaglo, 1990¹¹). The key factor in population kinetics is the proportion of permanent grassland in a given landscape. If grassland cover diminishes in favour of arable land so do populations of common voles and on the inverse an increase of grassland is followed by an expansion of vole populations. Vole populations on cultivated land suffer from regular extinctions due to agricultural operations and an increased risk of predation.

⁶ Rinke, T. (1990) Zur Nahrungsökologie von *Microtus arvalis* (Pallas, 1779) auf Dauergrünland. I: Allgemeine Nahrungspräferenzen. Z. Säugetierkunde 55, 106-114.

⁷ Rinke, T. (1991) Percentage of volume versus number of species: availability and intake of grasses and forbs in *Microtus arvalis*. Folia Zoologica 40 (2), 143-151.

⁸ Schröpfer, R. and Hildenhagen, U. (1984) Die Säugetiere Westfalens. Feldmaus - *Microtus arvalis* (Pallas, 1779). Abhandlungen aus dem Westfälischen Museum für Naturkunde, 46(4): 204-215.

⁹ Delattre, P., Giraudoux, P., Baudry, J., Musard, P., Toussaint, M., Truchetet, D., Stahl, P., Poule, M. L., Artois, M., Damange, P. And Quere, J.-P. (1992) Land use patterns and types of common vole (*Microtus arvalis*) population kinetics. Agriculture, Ecosystems and Environment 39: 153-169.

¹⁰ Jaworska, K., F. Polensy, W. Muller, and R. W. Olszak. (1995) The cover of herbaceous plants in an IPM apple orchard and its influence on the occurrence of rodents. Acta Horticulturae 422:431-432.

¹¹ Mackin-Rogalska, R. and Nabaglo, L. (1990) Geographical variation in cyclic periodicity and synchrony in the common vole, *Microtus arvalis*. Oikos 59: 343-348.

*Therefore, since in ornamental nurseries the vegetative undergrowth will be managed, the assumption that voles would forage 100% of their time in these crops is considered overly conservative. In reality, based on the preferred habitat and population dynamics of the vole as described above, ornamental nurseries are not considered to be an attractive area for foraging. It is clear that voles require permanent and undisturbed vegetation cover and that the common agricultural practices within the proposed crop, i.e. the extensive use of herbicides and regular maintenances (e.g., mowing, placing ground tarps, potting the plants) would significantly minimise the presence of weeds / grasses; therefore reducing any potential feed items that a small herbivorous mammals would seek in the treated area. Furthermore, in many regions voles are regarded as pests in nurseries and are actively controlled. However, the **PT has been retained as 1** because no quantifiable data are available for ornamentals, even though it is clear that this is a worst-case assumption.*

C) *Deposition factor*

*The diet of the vole is assumed to be 100% grass and the crop itself is not an attractive food source. For the relevant scenario “BBCH 40-49” no interception of grass/weeds by the crop is assumed in the EFSA Bird and Mammal Guidance Document (2009). However, this is considered to be unrealistic assumptions and it is more appropriate to assume interception of 60%, in line with the FOCUS Guidance Document on Ground Water (2011) for plants with a similar structure (e.g. strawberries) at BBCH 40 - 89. Thus a **deposition factor (DF) of 0.4** has been applied to the risk assessment.*

Refined TER values for small herbivorous mammal, vole

The refined acute and chronic TER values for small herbivorous mammals, e.g., vole, are presented in tables below, with consideration to the refinements discussed above.

Refined acute TER value for voles foraging in treated ornamentals (application rate: 5 x 4.25 kg a.s./ha with 7 day interval)

<i>Crop/scenario</i>	<i>Shortcut value</i>	<i>MAF</i>	<i>DF</i>	<i>DDD (mg/kg bw)</i>	<i>Endpoint (mg/kg bw)</i>	<i>TER</i>	<i>Tigger value</i>
Ornamentals BBCH 40-49	136.4	1.9	0.4	441	> 5000	11.3	10

^c DF: deposition factor (from EFSA 2009)

MAF: multiple application factor

DDD: daily dietary dose

Refined chronic TER value for voles foraging in treated ornamentals (application rate: 5 x 4.25 kg a.s./ha with 7 day interval)

<i>Representative species</i>	<i>FIR /bw^a</i>	<i>Food type</i>	<i>RUD^b</i>	<i>PT</i>	<i>PD</i>	<i>f_{twa}</i>	<i>MAF</i>	<i>DF^c</i>	<i>DDD (mg/kg)</i>	<i>DDD sum</i>	<i>End-point (mg/kg bw)</i>	<i>TER</i>	<i>Tigger value</i>
Small herbivorous mammals "vole"	1.33	Monocot plants	54.2	1	0.35	0.53	2.4	0.4	54.56	119.9	1200	10.0	5
	1.62	Dicot plants	28.7	1	0.65				65.35				

^a FIR/bw: food intake rate per body weight for mixed diet was calculated according to EFSA (2009), Appendix G

^b RUD: residues per unit dose (from EFSA 2009)

^c DF: deposition factor (from EFSA 2009)

MAF: multiple application factor

TWA: time weighted average factor

PT: proportion of diet obtained in the treated area

PD: proportion of food type in the diet

DDD: daily dietary dose

RMS comments on Notifier's proposal of refined long-term risk assessment:

RMS agrees to use the common vole (*Microtus arvalis*) as a specific focal species. It is identified in EFSA GD (2009) as the representative species for ornamentals with canopy directed application and it is a widespread and common species throughout the Europe. Its primary habitats are meadows, forest steppe, fallow lands etc. The species is frequent in agricultural fields, orchards, vineyard. It is considered sufficiently protective also for other species due to its low body weight.

Ad A): At the Pesticides Peer Review 149 Experts' Meeting on Ecotoxicology (23 - 27 October 2016), it was agreed to use PD 0.24 for grass and 0.76 for non-grass herbs in food of common vole, based on paper by Rinke (1991). This PD refinement can be used for spring and summer application (this is the case of daminozide) and for long-term risk only.

Ad B): RMS agrees to use PT of 1.

Ad C): RMS agrees with using of refined deposition factor of 0.4 in the risk assessment. Although ornamentals represent a wide range of plant species, the interception of 60% is considered worst-case for most of crops in BBCH 40-49. However, there is a small uncertainty that the crop itself could be consumed by voles as well.

The TER calculation using PT and RUD values proposed by RMS is presented below.

Table B.9.2.2-3 Refined acute TER values for small herbivorous mammal (common vole) foraging in treated ornamentals

Specific focal species / Scenario	Shortcut value	PD	PT	MAF	Deposition factor	AR (kg a.s./ha)	DDD (mg/kg bw)	End-point (mg/kg bw)	TER _A	Trigger value
Field use (application rate: 5 x 4.25 kg a.s./ha with 7 day interval)										
Common vole / BBCH 40-49	136.4	1	1	1.9	0.4	4.25	441	>5000	>11.34	10
Protected use (application rate: 5 x 7.65 kg a.s./ha with 7 day interval)										
Common vole / BBCH 40-49	136.4	1	1	1.9	0.4	7.65	793	>5000	>6.31	10

MAF: multiple application factor

PT: proportion of diet obtained in the treated area

PD: proportion of food type in the diet

AR: single application rate

DDD: daily dietary dose

Value(s) in bold are below the trigger value

Table B.9.2.2-4 Refined long-term TER values for small herbivorous mammal (common vole) foraging in treated ornamentals

treated ornamentals													
Specific focal species / Scenario	FIR / bw ^a	Food type	Mean RUD ^b	PT	PD	MAF / DF	f _{TWA}	AR (kg a.s./ha)	DDD (mg/kg bw)	DDD sum	End-point (mg/kg bw/d)	TER _{LT}	Trigger value
Field use (application rate: 5 x 4.25 kg a.s./ha with 7 day interval)													
Common vole / BBCH 40-49	1.33	Grass	54.2	1	0.24	2.4	0.53	4.25	37.41	113.82	500	4.39	5
	1.62	Non-grass	28.7		0.76	0.4			76.41				
Protected use (application rate: 5 x 7.65 kg a.s./ha with 7 day interval)													
Common vole / BBCH 40-49	1.33	Grass	54.2	1	0.24	2.4	0.53	7.65	67.34	204.88	500	2.44	5
	1.62	Non-grass	28.7		0.76	0.4			137.54				

^a FIR/bw: food intake rate per body weight according to EFSA (2009)

^b RUD: residues per unit dose according to EFSA (2009)

DF: deposition factor

MAF: multiple application factor

f_{TWA}: time weighted average factor

PT: proportion of diet obtained in the treated area

PD: proportion of food type in the diet

AR: single application rate

DDD: daily dietary dose

Value(s) in bold are below the trigger value

All the TER values, except for acute risk for field use remained below the relevant triggers. No further refinement was available. Therefore, the high dietary long-term risk for field use and the high dietary acute and long-term risk for protected use (other than permanent greenhouses) has been concluded for small herbivorous mammal (common vole) for BBCH 40-49.

Dietary risk to mammals from metabolites

No studies on residues in plants were available.

As methanol was identified as a potentially relevant metabolite in surface water and soil compartments, potential exposure of mammals to this metabolite should be assessed. No toxicity data were available for the metabolite methanol.

However, based on the physical-chemical properties of methanol; i.e. high vapour pressure (1.69×10^4 Pa at 25°C) from soil, it is assumed that methanol will be present in relevant food items in rather small amounts. Therefore, the exposure of mammals to methanol is expected to be negligible and the risk is considered to be covered with the risk assessment for the parent daminozide. No special dietary risk assessment for methanol is required.

Exposure to mammals will be predominately dietary, through the consumption of residue on food items e.g. sprayed crop, weeds and insects. There is also a possibility that mammals can be exposed via drinking water, see section below on exposure via drinking water.

Risk assessment for drinking water exposures

The risk assessment for drinking water exposures follows the guidance of the EFSA Journal 2009 7(12):1438 Section 5.5.

The guidance document states that the leaf scenario is needed for leafy vegetables forming heads and for other leafy vegetables (e.g. cauliflower) where the morphology facilitates the collection of rain or irrigation water. The uses of the daminozide do not include such crops. Therefore, the leaf scenario is not relevant for this assessment. The relevant scenario for the proposed uses is the puddle scenario, which is relevant for all outdoor uses.

According to the EFSA guidance document an assessment for puddle scenario is not required when the ratio of effective application rate (in g/ha) to the relevant endpoint (in mg/kg bw/d) does not exceed 50 in the case of less sorptive substances ($K_{oc} < 500$ L/kg) or 3000 where the $K_{oc} \geq 500$ L/kg.

Daminozide has a K_{oc} of 26.6 L/kg (mean). The maximum effective rate of use of Dazide Enhance is calculated for field use (5 x 4.25 kg a.s./ha with a 7 day interval) and for protected use other than greenhouses (5 x 7.65 kg a.s./ha with a 7 day interval) for ornamentals. The effective application rate is calculated by multiplying the proposed application rate by a MAF value based on the DT_{50} in soil (EFSA, 2009) for the active substance; for daminozide the maximum soil DT_{50} is 0.37 days.

Methanol has a K_{oc} of 1.0 L/kg. For the calculation of methanol PECs it was assumed that daminozide was instantly degraded, and therefore daminozide application rates were corrected for the relative molecular masses of

parent and metabolite, as well as for the maximum formation of methanol. Therefore the maximum effective rate of methanol is calculated for field use (5 x 4.25 kg a.s./ha with a 7 day interval) and for protected use other than greenhouses (5 x 7.65 kg a.s./ha with a 7 day interval) for ornamentals. The effective application rate is calculated by multiplying the proposed application rate by a MAF value based on the DT₅₀ in soil for the metabolite; for methanol the soil DT₅₀ is 3.9 days (geomean; calculated). Note that the soil DT₅₀ is considered to be conservative since exposure to the metabolite is expected to be limited based on the physical-chemical properties of methanol; i.e. high vapour pressure (1.69 x 10⁴ Pa at 25°C) from soil.

The table below summarises the ratios for daminozide and its metabolite methanol using both the acute and long-term endpoints.

Table B.9.2.2-5 Ratios of effective application rate to endpoints for daminozide and its metabolite

Test substance	Time scale	Application rate (g a.s./ha)	MAF	Effective application rate (g a.s./ha)	Endpoint	Ratio	Trigger value
Daminozide	Field use (application rate: 5 x 4.25 kg a.s./ha with 7 day interval)						
	Acute	4250	1.00 ^a	4250	>5000 mg/kg bw	<0.85	50
	Long-term				500 mg/kg bw/d	8.50	
	Protected use (application rate: 5 x 7.65 kg a.s./ha with 7 day interval)						
	Acute	7650	1.00 ^a	7650	>5000 mg/kg bw	<1.80	50
	Long-term				500 mg/kg bw/d	1.53	
Methanol	Field use (application rate: 5 x 4.25 kg a.s./ha with 7 day interval)						
	Acute	4250	1.40 ^c	5950	>500 mg/kg bw ^d	<11.9	50
	Long-term				50 mg/kg bw/d ^e	119	
	Protected use (application rate: 5 x 7.65 kg a.s./ha with 7 day interval)						
	Acute	7650	1.40 ^c	10710	>500 mg/kg bw ^d	<21.42	50
	Long-term				50 mg/kg bw/d ^e	214	

^a Based on the geomean soil DT₅₀ of 0.12 days

^c Based on the soil DT₅₀ of 3.9 days (geomean)

^d There are no toxicity data available for the metabolites methanol, therefore the amebolite has been assumed to be 10 times more toxic than the parent (LD₅₀ >5000 mg a.s./kg bw / 10 = >500 mg a.s./kg bw).

^e There are no toxicity data available for the metabolite methanol, therefore the amebolite has been assumed to be 10 times more toxic than the parent (NOEL = 500 mg a.s./kg bw/d / 10 = 50 mg a.s./kg bw/d).

MAF: Multiple application factor

The above ratios for daminozide are below the trigger value of 50 indicating an acceptable risk to mammals *via* drinking water contaminated from the proposed use of daminozide. However, the long-term ratios for methanol are above the trigger value and a Tier 1 drinking water assessment is required.

The long-term drinking water assessment for methanol is presented in the table below. The default drinking water rate (DWR) given in EFSA's Bird and Mammals Guidance (2009) has been used, along with the calculated PEC_{puddle} , and toxicity endpoints to calculate the TER.

Table B.9.2.1-6 Tier I drinking water assessment (puddle scenario) for the proposed use of Dazide Enhance

Test substance	Generic spp.	Time-scale	DWR (L/kg bw/d)	PEC_{puddle} (mg a.s./L)	Daily dose (mg a.s./kg bw)	Endpoint (mg a.s./kg bw/d)	TER	Trigger value
Methanol	Field use (application rate: 5 x 4.25 kg a.s./ha with 7 day interval)							
	Small granivorous mammal	Long-term	0.24	19.77	4.74	50 ^a	10.55	5
	Protected use (application rate: 5 x 7.65 kg a.s./ha with 7 day interval)							
	Small granivorous mammal	Long-term	0.24	35.16	8.44	50 ^a	5.92	5

^aThere are no toxicity data available for the metabolite methanol, therefore the am metabolite has been assumed to be 10 times more toxic than the parent (NOEL = 500 mg a.s./kg bw/d / 10 = 50 mg a.s./kg bw/d).

The above TER values for metabolite methanol are above the trigger value of 5, demonstrated low risk *via* drinking water.

Risk for Bioaccumulation and Secondary Poisoning

Substances with a high bioaccumulation potential could theoretically bear a risk of secondary poisoning for mammals if feeding on contaminated prey like fish or earthworms. For organic chemicals, a $\log P_{\text{OW}} > 3$ is used to trigger an in-depth evaluation of the potential for bioaccumulation.

As the $\log P_{\text{OW}}$ of daminozide and methanol are less than the trigger value of 3 ($\log P_{\text{OW}}$ at pH 7 = -1.5 and -0.77¹², respectively), the risk to mammals from secondary poisoning is considered to be negligible and no further consideration is required.

Conclusion – risk to vertebrates other than birds

No acute risks and no reproductive risks from drinking water exposure and secondary poisoning were identified for mammals for field use.

No acute and reproductive risks were identified for mammals for protected use in permanent greenhouses.

High dietary reproductive risk was concluded for small herbivorous mammal scenario (common vole) for field use.

High dietary acute and reproductive risk was concluded for small herbivorous mammal scenario (common vole) for protected use (other than permanent greenhouses).

No risks were identified for methanol.

¹² Material Safety Data Sheet – Methanol (CAS # 67-56-1). <https://fscimage.fishersci.com/msds/14280.htm>

B.9.3 Effects on aquatic organisms**B.9.3.1 Acute toxicity to fish, aquatic invertebrates, or effects on aquatic algae and macrophytes****B.9.3.1.1 Acute toxicity to fish****i) Acute toxicity of Dazide Enhance to common carp (*Cyprinus carpio*)**

Reference:	(2009) Acute Toxicity to Common Carp (<i>Cyprinus carpio</i>)
Report No.:	0673/0006
Guideline:	OECD 203 (1992); Method C.1 of Commission Regulation (EC) No. 440/2008
GLP:	Yes
Previous evaluation:	Submitted for the purpose of renewal

Executive Summary:

The objective of this study was to investigate the acute toxicity of the test item to common carp.

Following a preliminary range-finding test (1.0, 10, 100 and 1000 mg test item/L), fish were exposed, in groups of seven, to an aqueous solution of the test item over a range of concentrations of 0, 100, 180, 320, 560 and 1000 mg/L for a period of 96 hours under semi-static conditions. The number of mortalities and any sub-lethal effects of exposure in each vessel were determined 3 and 6 hours after the start of exposure and then daily throughout the test until termination after 96 hours.

In a separate positive control experiment, common carp were exposed to an aqueous solution of the reference item at concentrations of 0.1, 0.18, 0.32, 0.56 and 1.0 mg/L for 96 hours under semi-static conditions.

Samples for analytical confirmation of exposure concentrations were taken at 0, 24, 48, 72 and 96 hours of exposure. The measured test item concentrations ranged from 80% – 101% of nominal. Therefore, the effect parameters are expressed in terms of analytically confirmed nominal concentrations.

The 96h-LC₅₀ of the test item to common carp based on nominal test concentrations was 420 mg/L with 95% confidence limits of 320 – 560 mg/L. The NOEC value was 320 mg/L.

The 96h-LC₅₀ for the reference item to common carp based on nominal concentrations was 0.42 mg/L with 95% confidence limits of 0.32 – 0.56 mg/L. The NOEC value was 0.18 mg/L.

Material and methods:**A. MATERIALS**

1. Test material	Dazide Enhance SG
Description:	White granules
Batch no.:	1401107012

Active substance:	Daminozide
Content:	84.9% w/w
Control:	Test water without test substance
Reference item:	Pentachlorophenol, sodium salt

2. Test animals

Species:	Common carp (<i>Cyprinus carpio</i>)
Source:	██ ██████████
Number:	7 per vessel
Loading rate:	1.01 g bodyweight/L (based on mean weight value)
Age:	Juvenile
Acclimatisation:	13 days
Feeding:	The animals were not fed during the test

B. STUDY DESIGN AND METHODS

1. Test design

System:	Semi-static system
Duration:	96 hours
Test vessel:	20 L glass exposure vessel
Test medium:	Dechlorinated and partly softened tap water
Concentrations:	100, 180, 320, 560 and 1000 mg/L

2. Environmental conditions

Oxygen content:	7.7 – 9.2 mg/L (85 – 101% saturation)
Temperature:	20 - 22°C
pH:	4.2 – 8.1
Photoperiod:	16-h light, 8-h darkness with 20 minute transition period

3. Dose preparations

Amounts of test item (2.0, 3.6, 6.4, 11.2 and 20.0 g) were each separately dispersed directly in 20 litres of dechlorinated tap water and stirred to give the 100, 180, 320, 560 and 1000 mg/L test concentrations respectively.

4. Animal assignment and treatment

At the start of the test 7 fish were placed in each test and control vessel at random. The vessels were then covered to reduce evaporation and maintained in a temperature controlled room. The fish were exposed to the test item for 96 hours. The vessels were aerated via narrow bore glass tubes. The fish received no food during exposure. A semi-static regime was employed involving a daily renewal of the test preparations to ensure that the concentrations of the test item remained near nominal and to prevent the build-up of nitrogenous waste products.

The fish had a mean standard length of 4.5 cm (sd = 0.3) and a mean weight of 2.88 g (sd = 0.61) at the end of the test. Based on the mean weight value this gave a loading rate of 1.01 g bodyweight/L.

In a separate positive control experiment, common carp were exposed to the reference item at concentrations of 0.1, 0.18, 0.32, 0.56 and 1.0 mg/L for 96-h.

5. Measurements/observations

Any mortalities and sub-lethal effects of exposure were recorded at 3, 6, 24, 48, 72 and 96 hours after the start of exposure. The criteria of death were taken to be the absence of both respiratory movement and response to physical stimulation.

Water temperature, pH and dissolved oxygen were recorded daily throughout the test.

Water samples were taken from the control and all surviving test groups at 0, 24, 48, 72 and 96 hours for quantitative analysis. Duplicate samples were taken and stored at -20°C for further analysis if necessary. The measured concentrations ranged from 80% - 101% of nominal value and so it was considered justifiable to calculate the LC₅₀ values in terms of the nominal concentrations only.

6. Statistics

The LC₅₀ values and associated confidence limits at 3, 6, 24, 48, 72 and 96-h were calculated by using the geometric mean method.

Results:

The study met the acceptability criteria prescribed by the protocol and was considered valid.

Observations of mortality and sub-lethal effects during the test are presented in Table ?? below.

Table B.9.3.1-1 Mortality and sub-lethal effects in acute toxicity study with Dazide Enhance

	Cumulative mortality (initial population = 7)						% Mortality	Sub-lethal effects
Nominal concentration (mg/L)	3-h	6-h	24-h	48-h	72-h	96-h	96-h	3 - 96-h
Control	0	0	0	0	0	0	0	No abnormalities detected
100	0	0	0	0	0	0	0	No abnormalities detected
180	0	0	0	0	0	0	0	No abnormalities detected
320	0	0	0	0	0	0	0	No abnormalities detected
560	0*	7	7	7	7	7	100	Moribund at 3-h

1000	7**	7	7	7	7	7	100	All dead at 3-h
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*After 3-h exposure all of the fish were observed to be moribund. Due to the approach of the substantial severity limit (Animals (Scientific Procedures) Act 1986) these fish were killed and classed as mortalities for the 6-h time point.

**After 1.5-h exposure all of the fish were observed to be dead. These fish were removed and classed as mortalities for the 3-h time point.

The results showed the highest concentration resulting in 0% mortality to 320 mg/L, the lowest concentration resulting in 100% mortality to be 560 mg/L and the NOEC to be 320 mg/L.

Sub-lethal effects of exposure were observed at the test concentration of 560 mg/L. This response was the presence of moribund fish.

Observations of the test solutions revealed the control to remain clear, the 100, 180 and 320 mg/L solutions to be clear with foam at the surface and the 560 and 1000 mg/L concentrations to be fine homogenous dispersions with foam at the surface.

As the test material was observed to form a dispersion in the test diluent, microscopic inspection was performed on the gill filaments of the dead fish. This examination revealed a thick layer of mucus covering the gills which meant it was not possible to determine if the test material had adhered to the gill filaments. After washing the gills it was revealed that the outer edges of the gills contained no blood and were breaking up. The pH of the 560 and 1000 mg/L concentrations was approximately 5 and 4 respectively therefore it was considered possible that this was a contributing factor to the observed effects of the gills rather than a physical effect of the test material.

Analysis of the test preparations at 24, 48, 72 and 96 hours showed measured test concentrations to range from 80% to 101% of nominal and so the results are based on nominal test concentrations only.

Table B.9.3.1-2 Verification of test concentrations

Sample	Nominal Concentration (mg/l)	Concentration Found (mg/l)	Expressed as a Percent of the Nominal Concentration (%)
0 hours	Control	<LOQ	-
	100	90.8	91
	180	166	92
	320	305	95
	560	568	101
	1000	978	98
24 hours Old Media	Control	<LOQ	-
	100	84.9	85
	180	163	91
	320	297	93
	560	540	97
	1000	955	95
24 hours Fresh Media	Control	<LOQ	-
	100	99.0	99
	180	169	94
	320	298	93
48 hours Old Media	Control	<LOQ	-
	100	88.3	88
	180	151	84
	320	289	90
48 hours Fresh Media	Control	<LOQ	-
	100	92.7	93
	180	167	93
	320	303	95
72 hours Old Media	Control	<LOQ	-
	100	80.4	80
	180	144	80
	320	263	82

Table B.9.3.1-2 Verification of test concentrations-continued

Sample	Nominal Concentration (mg/l)	Concentration Found (mg/l)	Expressed as a Percent of the Nominal Concentration (%)
72 hours Fresh Media	Control	<LOQ	-
	100	91.5	92
	180	162	90
	320	302	94
96 hours Old Media	Control	<LOQ	-
	100	85.7	86
	180	152	84
	320	290	91

The results of the positive control showed the highest test concentration resulting in 0% mortality to be 0.32 mg/L, the lowest concentration resulting in 100% mortality to be 0.56 mg/L and the NOEC to be 0.18 mg/L. These results were within the normal range for this reference material. The mean 96-h LC₅₀ value calculated from all positive control tests is 0.30 mg/L (sd = 0.08).

Conclusions:

The 96h-LC₅₀ of the test item to common carp based on nominal test concentrations was 420 mg/L with 95% confidence limits of 320 – 560 mg/L. The NOEC value was 320 mg/L.

RMS comments and conclusion:

The reported study is GLP compliant and was conducted according to OECD 203 guideline (1992). The test results are in compliance with the guideline's validity criteria (mortality control less than 10%; dissolved oxygen concentrations at least 60% of the air saturation value; concentrations of the test substance satisfactorily maintained throughout the test). The study is acceptable for regulatory use.

The 96-hour LC₅₀ is 420 mg Dazide Enhance SG/L (equivalent to 357 mg daminozide/L) and the 96-hour no-observed-effect concentration (NOEC) is 320 mg Dazide Enhance SG /L (equivalent to 272 mg daminozide/L), based on nominal concentrations.

ii) Acute toxicity of Dazide Enhance to common carp (*Cyprinus carpio*)

Reference:	(2010a) Acute Toxicity to Common Carp (<i>Cyprinus carpio</i>)
Report No.:	41004365
Guideline:	OECD 203 (1992); Method C.1 of Commission Regulation (EC) No. 440/2008
GLP:	Yes
Previous evaluation:	Submitted for the purpose of renewal

Executive Summary:

The objective of this study was to investigate the acute toxicity of the test item to common carp.

Following a preliminary range-finding test (1.0, 10 and 100 mg test item/L), fish were exposed, in groups of seven, to an aqueous solution of the test item over a range of concentrations of 0, 10, 18, 32, 56 and 100 mg/L for a period of 96 hours under semi-static conditions. The number of mortalities and any sub-lethal effects of exposure in each vessel were determined 3 and 6 hours after the start of exposure and then daily throughout the test until termination after 96 hours.

Samples for analytical confirmation of exposure concentrations were taken at 0, 24 and 96 hours of exposure. The measured test item concentrations ranged from 90% – 104% of nominal. Therefore, the effect parameters are expressed in terms of analytically confirmed nominal concentrations.

The 96h-LC₅₀ of the test item to common carp based on nominal test concentrations was 75 mg/L with 95% confidence limits of 56 – 100 mg/L. The NOEC value was 56 mg/L.

Materials and methods:**A. MATERIALS**

- | | |
|--------------------------|--|
| 1. Test material | FAL 2400 |
| Description: | White granular solid |
| Batch no.: | 4191010 XIII-NF2 |
| Expiry date: | October 2012 |
| Active substance: | Daminozide |
| Content: | 85.5% w/w |
| Control: | Test water without test substance |
|
 | |
| 2. Test animals | |
| Species: | Common carp (<i>Cyprinus carpio</i>) |
| Source: | ██ |
| Number: | 7 per vessel |
| Loading rate: | 1.30 g bodyweight/L (based on mean weight value) |
| Age: | Juvenile |
| Acclimatisation: | 13 days |
| Feeding: | The animals were not fed during the test |

B. STUDY DESIGN AND METHODS

- | | |
|-----------------------|--------------------|
| 1. Test design | |
| System: | Semi-static system |
| Duration: | 96 hours |

Test vessel:	20 L glass exposure vessel
Test medium:	Dechlorinated and partly softened tap water
Concentrations:	10, 18, 32, 56 and 100 mg/L

2. Environmental conditions

Oxygen content:	7.2 – 11.2 mg/L (77 – 123% saturation)
Temperature:	19 - 21°C
pH:	6.7 – 8.1
Photoperiod:	16-h light, 8-h darkness with 20 minute transition period

3. Dose preparations

An amount of test item (4000 mg) was dissolved in dechlorinated tap water and the volume adjusted to 1 litre to give a 4000 mg/L stock solution. Aliquots of this solution (50, 90, 160 and 280 mL) were each separately diluted to a final volume of 20 L of dechlorinated tap water, and stirred to give the 10, 18, 32 and 56 mg/L test concentrations respectively. A further 2000 mg of test item was dissolved in dechlorinated tap water and the volume adjusted to 500 mL to give a second 4000 mg/L stock solution. This stock was diluted in a final volume of 20 L of dechlorinated tap water and stirred, to give the 100 mg/L test concentration. Each of the stock solutions was inverted several times to ensure adequate mixing and homogeneity.

4. Animal assignment and treatment

At the start of the test 7 fish were placed in each test and control vessel at random. The vessels were then covered to reduce evaporation and maintained in a temperature controlled room. The fish were exposed to the test item for 96 hours. The vessels were aerated via narrow bore glass tubes. The fish received no food during exposure. A semi-static regime was employed involving a daily renewal of the test preparations to ensure that the concentrations of the test item remained near nominal and to prevent the build-up of nitrogenous waste products.

The fish had a mean standard length of 5.1 cm (sd = 0.1) and a mean weight of 3.72 g (sd = 0.40) at the end of the test. Based on the mean weight value this gave a loading rate of 1.30 g bodyweight/L.

5. Measurements/observations

Any mortalities and sub-lethal effects of exposure were recorded at 3, 6, 24, 48, 72 and 96 hours after the start of exposure. The criteria of death were taken to be the absence of both respiratory movement and response to physical stimulation.

Water temperature, pH and dissolved oxygen were recorded daily throughout the test.

Water samples were taken from the control and all surviving test groups at 0, 24 and 96 hours for quantitative analysis. The 0 and 24 hour samples were stored at -20 °C prior to analysis. Only test concentrations at the NOEC and above were analysed. Duplicate samples and samples at 24, 48 and 72 hours were taken and stored at -20°C for further analysis if necessary.

The measured concentrations ranged from 90% - 104% of nominal value and so it was considered justifiable to calculate the LC₅₀ values in terms of the nominal concentrations only.

6. Statistics

An estimate of the LC₅₀ values at 3, 6 and 24-h was given by inspection of the mortality data.

The LC₅₀ values and associated confidence limits at 48, 72 and 96-h were calculated by using the geometric mean method.

Results:

The study met the acceptability criteria prescribed by the protocol and was considered valid.

Observations of mortality and sub-lethal effects during the test are presented in Table below.

Table B.9.3.1-3 Mortality and sub-lethal effects in acute toxicity study with FAL 2400

	Cumulative mortality (initial population = 7)						% Mortality	Sub-lethal effects
Nominal concentration (mg/L)	3-h	6-h	24-h	48-h	72-h	96-h	96-h	3 - 96-h
Control	0	0	0	0	0	0	0	No abnormalities detected
10	0	0	0	0	0	0	0	No abnormalities detected
18	0	0	0	0	0	0	0	No abnormalities detected
32	0	0	0	0	0	0	0	No abnormalities detected
56	0	0	0	0	0	0	0	No abnormalities detected
100	0	0	0	7	7	7	100	Slight loss of equilibrium at 3-h. Loss of equilibrium at 6 and 24-h

The results showed the highest concentration resulting in 0% mortality to 56 mg/L, the lowest concentration resulting in 100% mortality to be 100 mg/L and the NOEC to be 56 mg/L.

Sub-lethal effects of exposure were observed at the test concentration of 100 mg/L. This response was loss of equilibrium.

Analysis of the test preparations at 0, 24 and 96 hours showed measured test concentrations to range from 90% to 104% of nominal and so the results are based on nominal test concentrations only.

Table B.9.3.1-4 Verification of test concentrations

Sample	Nominal Concentration (mg/l)	Concentration Found (mg/l)	Expressed as a Percentage of the Nominal Concentration
0 Hours (Fresh Media)	Control	<LOQ	-
	56	58.2	104
	100	99.4	99
24 Hours (Old Media)	Control	<LOQ	-
	56	56.1	100
	100	89.7	90
96 Hours (Old Media)	Control	<LOQ	-
	56	56.5	101

Conclusions:

The 96h-LC₅₀ of the test item to common carp (*Cyprinus carpio*) based on nominal test concentrations was 75 mg/L with 95% confidence limits of 56 – 100 mg/L. The NOEC value was 56 mg/L.

RMS comments and conclusion:

The reported study is GLP compliant and was conducted according to OECD 203 guideline (1992). The test results are in compliance with the guideline's validity criteria (mortality control less than 10%; dissolved oxygen concentrations at least 60% of the air saturation value; concentrations of the test substance satisfactorily maintained throughout the test). The study is acceptable for regulatory use.

The 96-hour LC₅₀ is 75 mg formulation/L (equivalent to 64 mg daminozide/L) and the 96-hour no-observed-effect concentration (NOEC) is 56 mg formulation /L (equivalent to 48 mg daminozide/L), based on nominal concentrations.

B.9.3.1.2 Acute toxicity to aquatic invertebrates**i) Acute toxicity of Dazide Enhance to *Daphnia magna* in a dose response test**

Reference:	Goodband, T.J. and Mullee, D.M. (2010b) FAL 2400: Acute Toxicity to <i>Daphnia magna</i>
Report No.:	41004366
Guideline:	OECD 202 (2004); Method C.2 of Commission Regulation (EC) No. 440/2008
GLP:	Yes
Previous evaluation:	Submitted for the purpose of renewal

Executive Summary:

The objective of this study was to determine the concentration of FAL 2400 estimated to immobilise 50% of the *Daphnia magna* (EC₅₀) after 24 and 48 hours exposure.

Following a preliminary range-finding test (1.0, 10 and 100 mg test item/L), twenty daphnids (2 replicates of 10 animals) were exposed to an untreated control or to an aqueous solution of the test item at concentrations of 10, 18, 32, 56 and 100 mg test item/L for 48 hours under static conditions. The number of immobilised *Daphnia* was recorded after 24 and 48 hours.

In a separate positive control experiment, *Daphnia magna* were exposed to an aqueous solution of the reference item at concentrations of 0.32, 0.56, 1.0, 1.8 and 3.2 mg/L for 48 hours under static conditions.

Samples for analytical confirmation of exposure concentrations were taken at the start and at the end of the test. The measured test item concentrations ranged from 95% – 109% of the nominal value. Therefore, the effect parameters are expressed in terms of analytically confirmed nominal concentrations.

The 48h-EC₅₀ for the test item to *Daphnia magna* based on nominal test concentrations was 60 mg/L with 95% confidence limits of 53 – 68 mg/L. The NOEC value was 32 mg/L.

The 48h-EC₅₀ for the reference item to *Daphnia magna* based on nominal concentrations was 0.75 mg/L with 95% confidence limits of 0.65 – 0.86 mg/L. The NOEC value was 0.32 mg/L.

Materials and methods:

A. MATERIALS

1.	Test material	FAL 2400
	Description:	White granular solid
	Batch no.:	4191010 XIII-NF2
	Expiry date:	October 2012
	Active substance:	Daminozide
	Content:	85.5% w/w
	Control:	Test medium without test substance
	Reference item:	Potassium dichromate
2.	Test animals	
	Species:	1 st instar <i>Daphnia magna</i>
	Source:	In-house laboratory culture
	Number:	20 (2 replicates of 10) per group
	Loading:	10 per vessel containing 200 mL of test solution
	Age:	< 24 hours old
	Feeding:	The animals were not fed during the test

B. STUDY DESIGN AND METHODS

1. Test design

System:	Static system
Duration:	48 hours
Test vessel:	250 mL glass jars
Test medium:	Reconstituted water
Concentrations:	10, 18, 32, 56 and 100 mg/L

2. Environmental conditions

Oxygen content:	8.9 – 9.2 mg/L (98 – 101% saturation)
Temperature:	20°C
pH:	6.1 – 8.1
Photoperiod:	16-h light, 8-h darkness with 20 minute transition period

3. Dose preparations

An amount of test item (200 mg) was dissolved in reconstituted water and the volume adjusted to 2 litres to give a 100 mg/L test concentration. Aliquots of this solution (50, 90, 160 and 280 mL) were separately added to reconstituted water and the volume adjusted to give the 10, 18, 32 and 56 mg/L test concentrations respectively. Each prepared concentration was inverted several times to ensure adequate mixing and homogeneity.

4. Animal assignment and treatment

At the start of the test 10 daphnids with an age of < 24 hours were placed in each test and control vessel at random. Duplicate vessels were used for each group. The vessels were then covered to reduce evaporation. The daphnids were exposed to the test item for 48 hours. The solutions were not aerated and the daphnids were not fed for the duration of the test period. The test preparations were not renewed during the exposure.

In a separate positive control experiment, daphids were exposed to the reference item at concentrations of 0.32, 0.56, 1.0, 1.8 and 3.2 mg/L for 48-h.

5. Measurements/observations

Observations of immobilisation or adverse reaction to exposure were recorded at 24 and 48 hours after test initiation. Immobile are those animals not able swim within 15 seconds after gentle agitation of the test vessel.

Water temperature was recorded daily throughout the test. Dissolved oxygen and pH were measured at the beginning and end of the test.

The concentration of the test item in the test preparations was verified by chemical analysis at 0 and 48 hours.

6. Statistics

An estimate of the EC₅₀ values was given by inspection of the immobilisation data at 24-h. The EC₅₀ value and associated confidence limits at 48-h and the slope of the response curve and its standard error were calculated by the trimmed Spearman-Kärber method (Hamilton *et al* 1977) using ToxCalc computer software package.

Results:

The study met the acceptability criteria prescribed by the protocol and was considered valid.

Observations of immobility during the test are presented in Table 10.2.2.2/01-1 below.

Table B.9.3.1-5 Immobilisation of *Daphnia* in acute toxicity study with FAL 2400

Solution (mg/L)	24 Hours		48 Hours	
	Immobilised		Immobilised	
	Total	Effect %	Total	Effect %
Control	0	0	0	0
FAL 2400				
10	0	0	0	0
18	0	0	19	95
32	0	0	0	0
56	4	20	8	40
100	5	25	20	100

After 48-h exposure, 95% immobilisation was observed at the test concentration of 18 mg/L. No immobilisation was observed in the 32 mg/L test concentration and only 40% immobilisation in the 56 mg/L test concentration therefore the observed immobilisation at 18 mg/L did not fit the immobilisation pattern. This immobilisation was therefore considered to be due to external contamination from an unknown source and was considered not to affect the integrity of the test.

Inspection of the immobilisation data at 24-h and analysis of the immobilisation data by the trimmed Spear-Kärber method at 48-h based on the nominal test concentrations gave the following results:

Time (h)	EC ₅₀ (mg/L)	95% Confidence limits (mg/L)
24	> 100	-
48	60	53 - 68

The NOEC after 24 and 48-h exposure was 32 mg/L.

Due to the unsuitable nature of the data it was not possible to calculate the slope and error of response curve at 24 and 48-h.

The reference item 24-h-EC₅₀ was 1.3 mg/L with a 95% confidence interval 1.1 – 1.5 mg/L. The 48-h-EC₅₀ was 0.75 mg/L with a 95% confidence interval 0.65 - 0.86 mg/L. These were within the range of the expected responses hence the animals are of suitable sensitivity.

The concentration of the test item in the test preparations was verified by chemical analysis at 0 and 48 hours. The measured concentrations ranged from 95% - 109% of nominal value and so it was considered justifiable to calculate the EC₅₀ values in terms of the nominal concentrations only. The results are presented in the table below.

Table B.9.3.1-6 Concentrations of the test substance in test media samples determined using HPLC-MS analysis

Sample	Nominal Concentration (mg/l)	Concentration Found (mg/l)	Expressed as a Percentage of the Nominal Concentration (%)
0 hours	Control	<LOQ	-
	10	10.5	105
	18	18.0	100
	32	30.3	95
	56	58.7	105
	100	104	104
48 hours	Control	<LOQ	-
	10	10.3	103
	18	18.0	100
	32	31.6	99
	56	61.1	109
	100	101	101

Conclusions

The 48h-EC₅₀ for the test item to *Daphnia magna* based on nominal test concentrations was 60 mg/L with 95% confidence limits of 53 – 68 mg/L. The NOEC value was 32 mg/L.

RMS comments and conclusion:

The reported study is GLP compliant and was conducted according to OECD 202 guideline (2004). The test results are in compliance with the guideline's validity criteria (mortality control less than 10%; dissolved oxygen concentrations at least 60% of the air saturation value). The study is acceptable for regulatory use.

OECD 202 TG recommends that the daphnids should be preferably grouped into 5 organisms per replicate while they were grouped into 10 organisms per replicate in the present study. However, the recommendation of the OECD 202 TG, that at least 2 ml of test solution should be provided for each animal, was fulfilled (20 ml test solution per daphnid was provided in the study).

RMS agrees with the study author that the immobilization of 95% at the test concentration of 18 mg/L was probably not treatment related since it did not fit the immobilisation pattern. No immobilisation was observed in the next higher concentration of 32 mg/L and only 40% immobilisation at the 56 mg/L test concentration.

The 48-hour EC₅₀ is 60 mg formulation/L (equivalent to 51 mg a.s./L) and the 48-hour no-observed-effect concentration (NOEC) is 32 mg formulation /L (equivalent to 27 mg a.s./L), based on nominal concentrations.

ii) Acute toxicity of Dazide Enhance to *Daphnia magna* in a limit test

Reference:	Hernádi, D. (2007a) Acute Immobilisation Test with Dazide Enhance SG (Dazide 85 WSG) on <i>Daphnia Magna</i>
Report No.:	07/482-023DA
Guideline:	OECD 202 (2004); U.S. EPA OPPTS 850.1010 (1996); Method C.2 of Commission Regulation (EC) No. 440/2008
GLP:	Yes
Previous evaluation:	Submitted for the purpose of renewal

Executive Summary:

The objective of this study was to determine the concentration of Dazide Enhance estimated to immobilise 50% of the *Daphnia magna* (EC₅₀) after 24 and 48 hours exposure. Young *Daphnia* were exposed in a static test to aqueous test medium containing the test item.

Following a preliminary range-finding test, twenty daphnids per group (5 per replicate, quadruplicate) were exposed to an untreated control and to nominally 100 mg/L Dazide Enhance, in a limit test. The total exposure period was 48 hours and samples for analytical confirmation of exposure concentrations were taken from the test group at the start and at the end of the test. Two samples were taken from the control group at the end of the test. The corresponding measured concentration was 101 mg/L at the start and 109 mg/L at the end of the test. As the deviation of measured concentrations from the nominal values was not higher than 20%, all reported results relate to the nominal concentration of the test item.

Under the conditions of this immobilisation study the 24h-EC₅₀ value was > 100 mg test item/L and the 48h-EC₅₀ value was > 100 mg test item/L. The 48h- LOEC value was > 100 mg test item/L and the 48-h NOEC value was 100 mg test item/L.

Materials and methods:

A. MATERIALS

1. Test material	Dazide Enhance
Description:	White, free flowing granule
Batch no.:	OXX0608005
Expiry date:	August 2009

Active substance:	Daminozide
Content:	85% w/w
Control:	Test medium without test item
Reference item:	Potassium dichromate

2. Test animals

Species:	<i>Daphnia magna</i>
Source:	National Institute of Public Health, 1097 Budapest Gyáli u. 2-6. Hungary
Number:	20 in the test group and 20 in the control group, divided into four replicates (5 animals / replicate)
Loading:	5 per vessel containing 40 mL of test solution
Age:	< 24 hours old
Acclimatisation:	None as the water used was similar to the culture water
Feeding:	The animals were not fed during the test

B. STUDY DESIGN AND METHODS

1. Test design

System:	Static system
Duration:	48 hours
Test vessel:	50 mL glass beaker
Test medium:	Iso medium
Concentration:	100 mg/L (nominal), 105 mg/L (average measured)

2. Environmental conditions

Oxygen content:	6.5 – 6.9 mg/L
Temperature:	20.1 – 20.2°C
pH:	6.37 – 7.80
Photoperiod:	Artificial illumination, 16-h light and 8-h dark

3. Dose preparation

The test item was dissolved into test water as homogeneously as possible by intense stirring and by means of ultrasonic treatment. No solvent substance was used.

4. Animal assignment and treatment

Daphnids with an age <24 hours, were introduced into the test solutions immediately after their preparation.

Four replicate test vessels were prepared each for the test item and the control groups, with five daphnids per vessel. Each vessel contained 40 mL of test medium. The daphnids were exposed to the test item for 48 hours. The daphnids were not fed for the duration of the test period.

5. Measurements/observations

The number of mobile and immobilised test animals was observed and recorded 24 and 48 hours after the start of the test.

The oxygen concentrations and pH of the controls and the test solutions were measured at the beginning and at the end of the test. The water temperature was measured daily.

For determination of the test item concentration, samples were taken from each test group at the start and from each replicate at the end of the test. Two samples were taken from the control group at the end of the test. As the deviation of measured concentrations from the nominal values was not higher than 20%, all reported results are related to the nominal concentration of the test item.

6. Statistics

The 24 and 48h-EC₅₀ could not be determined because the test substance appeared to be not toxic.

Results:

The measured test item concentration was 101 mg/L at the start and 109 mg/L at the end of the test.

The study met the acceptability criteria prescribed by the protocol and was considered valid.

Observations of immobility during the test are presented in Table below.

Table B.9.3.1-7 Immobilisation of *Daphnia* in acute toxicity study with Dazide Enhance

Solution (mg/L)	24 Hours		48 Hours	
	Immobilised		Immobilised	
	Total	Effect %	Total	Effect %
Control	0	0	0	0
Dazide Enhance 100	0	0	0	0

No immobilisation was observed in any of the groups tested during the exposure period.

Conclusions:

Under the conditions of this immobilisation study the 24h-EC₅₀ value was > 100 mg test item/L and the 48h-EC₅₀ value was > 100 mg test item/L. The 48h- LOEC value was > 100 mg test item/L and the 48-h NOEC value was 100 mg test item/L.

As the 48-h EC₅₀ for Dazide enhance SG is higher than 100 ppm, the test item can be interpreted as practically non-toxic. Dazide Enhance SG does not meet the criteria for classification according to the EU labelling regulations Commission Directive 2001/59/EC for classification and labelling of dangerous goods.

RMS comments and conclusion:

The reported study is GLP compliant and was conducted according to OECD 202 guideline (2004) and OPPTS 850.1010 (1996). The test results are in compliance with the guidelines' validity criteria (mortality control less than 10%; dissolved oxygen concentrations at least 60% of the air saturation value) . The study is acceptable for regulatory use.

The 48-hour EC₅₀ is > 100 mg mg formulation/L (equivalent to >85 mg a.s./L) and the 48-hour no-observed-effect concentration (NOEC) is 100 mg formulation /L (equivalent to 85 mg a.s./L), based on nominal concentrations.

B.9.3.1.3 Effects on aquatic algae and macrophytes

i) Effects of Dazide Enhance on freshwater green alga *Pseudokirchneriella subcapitata*

Reference:	Hernádi, D. (2007b) Dazide Enhance SG (Dazide 85 WSG) growth inhibition test on algae (<i>Pseudokirchneriella subcapitata</i>)
Report No.:	07/482-022AL
Guideline:	OECD 201 (2006); U.S. EPA OPPTS 850.5400 (1996); Method C.3 of Commission Directive 92/69/EEC
GLP:	Yes
Previous evaluation:	Submitted for the purpose of renewal

Executive Summary:

The effect of Dazide Enhance SG (Dazide 85 WSG) was assessed to determine the inhibition of algal growth (E_rC₅₀, E_bC₅₀ and E_yC₅₀) using the unicellular green alga *Pseudokirchneriella subcapitata*, over an exposure period of 72 hours under static conditions. Nominal test concentrations were 6.25, 12.5, 25, 50 and 100 mg test item/L. An untreated control group was tested in parallel. The test design included three replicates per test concentration and six replicates for the untreated control. Algal growth was determined by cell counts at 24 hour interval.

Results of the analytical measurements from samples taken at the start and end of the exposure demonstrated that the test item was stable (deviations from nominal concentrations were <20%). With respect to the inhibitory effect of the test item, the 0-72 hour average growth rates, the 0-72 hour areas under the growth curve and the 0-72 hour yield were not statistically different from that of the control group in any test concentration. Therefore, the overall

EC₅₀ is > 100 mg test item/L, the NOEC is 100 mg test item/L, and the LOEC is > 100 mg test item/L after 72 hours. Endpoints based on nominal test concentration.

Materials and methods:

A. MATERIALS

1. **Test material:** Dazide Enhance SG (Dazide 85 WSG)
Description: White free flowing granules
Lot/Batch: OXX0608005
Purity: 85%
2. **Reference item:** Potassium dichromate (K₂Cr₂O₇)
(Tested in a separate study (study code 07/421-022AL))
3. **Vehicle:** Algal Mineral salts Test Medium according to OECD 201 without Na₂EDTA x 2H₂O

B. STUDY DESIGN AND METHODS

1. **Test organism:** *Pseudokirchneriella subcapitata* (unicellular green alga) (Printz-Starr)
Growth stage: Inoculum - four days since previous transfer
Source: Georg-August-Universität Göttingen, Albrecht-von-Haller-Institut für Pflanzenwissenschaften
Experimentelle Phykologie und sammlung von Algenkulturen (SAG), Göttingen, Germany
Initial cell density: 1.0 x 10⁴ cells/mL
2. **Growth medium:** Algal Mineral salts Test Medium according to OECD 201 without Na₂EDTA x 2H₂O
pH: Not reported
3. **Test concentrations:** Nominal: 6.25, 12.5, 25.0, 50.0 and 100.0 mg test item/L
Reference item: 0.25, 0.50, 1.00, 2.00 and 4.00 mg/L (*separate test*)
4. **Test vessels:** 250 mL glass flasks, containing 100 mL of test solution
5. **Environmental conditions:**
Temperature: 21.8-23.8°C
pH: 6.21-8.74 at test start, 8.56-10.24 at test termination
Photoperiod: Continuous illumination (8213.97 lux)
Shaking rate: Shaking speed not reported
6. **Test organism set up and treatment:**

Based on the results of a preliminary test, nominal concentrations ranging from 6.25 to 100 mg test item/L were selected for the definitive test. Cultures of *Pseudokirchneriella subcapitata* (initial cell density of 1×10^4 cells/mL) in replicates of 3 per test concentration and 6 replicates of untreated test medium were evaluated for algal inhibition growth during a 72 hour exposure period under static conditions.

7. Dose preparation:

A stock solution with a concentration of 100 mg/L was prepared with test item and test medium. The test solutions were prepared from diluting this stock solution to obtain the desired test concentrations.

8. Measurements and observations:

The cell concentrations were determined at 24, 48 and 72 hours after test initiation by microscopic method.

pH was recorded at the start and end of the study. Water temperature was checked at the start and at each 24 hours, but continuous measurements were taken with a min/max thermometer inside the climate chamber. Light intensity was checked once during the study.

For determination of the test item concentrations, samples were taken from each test group at the start and from each replicate at the end of the study. From the control group two samples were taken at the end of the test. Samples were measured by HPLC.

9. Statistics:

Statistical comparison of average growth rates, areas under the growth curves and yield in untreated control and in treated groups were carried out using analysis of variance (ANOVA) and Bonferroni t-Test ($\alpha = 0.05$) by statistical software program Toxstat (Western EcoSystems Technology).

Probit analysis was not deemed appropriate as no inhibition $\geq 50\%$ occurred in any test item concentration.

Results:

A. BIOLOGICAL EFFECTS:

With respect to the inhibitory effect of the test item, the 0-72 hour average growth rates (Table CA 10.2.1.3/01-01), the 0-72 hour areas under the growth curve (Table 10.2.1.3/01-02) and the 0-72 hour yield (Table 10.2.1.3/01-03) were not statistically different from that of the control group in any test concentration.

Table B.9.3.1-8 Growth rates (μ) and percentage inhibition of μ during the study period

Concentration (mg/L)	Growth rate (μ) and % inhibition of μ					
	0-24 hrs		0-48 hrs		0-72 hrs	
	μ	%	μ	%	μ	%
Control	0.0578	0.0	0.0588	0.0	0.0569	0.0
6.25	0.0538	6.9	0.0577	1.7	0.571	-0.3
12.5	0.0538	6.9	0.0577	1.7	0.571	-0.2
25	0.0578	0.0	0.0573	2.6	0.576	-1.2
50	0.0538	6.9	0.0559	4.9	0.569	0.0
100	0.0498	13.8	0.0554	5.7	0.0572	-0.5

Table B.9.3.1-9 Area under the growth curves (A) and percentage inhibition of A during the study period

Concentration (mg/L)	Ares under growth curves (A) and % inhibition of A					
	0-24 hrs		0-48 hrs		0-72 hrs	
	A	%	A	%	A	%
Control	36	0.0	262	0.0	1166	0.0
6.25	32	11.1	244	6.9	1148	1.5
12.5	32	11.1	244	6.9	1144	1.9
25	36	0.0	248	5.3	1172	-0.5
50	32	11.1	228*	13.0	1104	5.3
100	28	22.2	216*	17.6	1104	5.3

* Statistically significant different compared to the control values (Bonferroni t-Test, $\alpha = 0.05$)

Table B.9.3.1-10 Yield (Y) and percentage inhibition of Y during the study period

Concentration (mg/L)	Yield (Y) and % inhibition of Y	
	0-72 hrs	
	Y	%
Control	59.5	0.0
6.25	60.3	-1.4
12.5	60.0	-0.84
25	62.3	-4.76
50	59.3	0.28
100	60.7	-1.96

B. TOXICITY ENDPOINTS:

As there were no statistically significant differences observed in any of the test parameters after 72 hours, the overall toxicity values are: $EC_{50} > 100$ mg test item/L, $NOEC = 100$ mg test item/L and the $LOEC$ is > 100 mg test item/L after 72 hours (based on nominal concentrations).

The Reference item demonstrated the validity of the study with the following toxicity endpoints: $E_rC_{50} = 0.99$ mg/L (95% CI 0.81-1.21 mg/L), $E_bC_{50} = 0.66$ mg/L (95% CI 0.52-0.84 mg/L), and $E_yC_{50} = 0.48$ mg/L (95% CI 0.36-0.64 mg/L)

C. ANALYSIS:

The analysed concentrations varied between 105 and 107% of the nominal concentration at the start of the study and between 107 and 111% at the end of the test. Results are summarised in Table 10.2.1.3/01-04. As the deviations from the nominal concentration were less than 20% in the analysed concentrations during the study, the results are based on nominal concentrations.

Table B.9.3.1-11 Measured concentrations of test item

Nominal concentration (mg test item/L)	Measured concentration (mg test item/L)			% change (0-72 hrs)
	start	end	mean	
6.25	6.62 (106%)	6.96 (111%)	6.79	-8.64
12.5	13.1 (105%)	13.5 (108%)	13.3	-6.4
25	26 (106%)	27 (107%)	26.5	-6.0
50	53 (107%)	54 (108%)	53.5	-7.0
100	106 (106%)	109 (109%)	107.5	-7.5

Conclusion:

With respect to the inhibitory effect of the test item, the 0-72 hour average growth rates, the 0-72 hour areas under the growth curve and the 0-72 hour yield were not statistically different from that of the control group in any test concentration. Therefore, the overall EC_{50} is > 100 mg test item/L, the NOEC is 100 mg test item/L, and the LOEC is > 100 mg test item/L after 72 hours (based on nominal concentrations).

RMS comments and conclusion:

The reported study is GLP compliant and was conducted according to OECD 201 (2006) and OPPTS 850.5400 (1996) guidelines.

To check the guidelines' validity criteria, the coefficients of variation of average specific growth rates in control replicates were calculated and are presented in the table below:

Table B.9.3.1-12 Average coefficient of variance at 0-72 hours and section-by-section in the control cultures

Replicates	0-72 h			Section by section (day 0-1, 1-2, 2-3)			
	Average growth rate (day ⁻¹)	St Dev	CV (%)	Average growth rate (day ⁻¹)	St Dev	CV (%)	Mean CV (%)
A	1.37	0.029	2.10	1.32	0.12	9.10	7.16
B				1.38	0.13	9.10	
C				1.35	0.11	8.38	
D				1.39	0.17	12.40	
E				1.40	0.018	1.27	
F				1.36	0.037	2.73	

According to current OECD 201 guideline the validity criteria were met (the specific growth rate in control was 1.37 per day within the 72-hour period (should be greater than 0.92 per day); the mean coefficient of variation for section-by section specific growth rates in the control cultures was 7.16% (should be less than 35%); the coefficient of variation of average specific growth rates during the whole test period in replicate control cultures was 2.10 (should be less than 7%)).

The study is acceptable for regulatory use.

The 72-hour E_rC₅₀, E_bC₅₀ and E_yC₅₀ is >100 µg daminozide/L, the 72-hour no-observed-effect concentration (NOEC) is 100 µg daminozide/L, based on nominal concentrations.

B.9.4 Risk assessment for aquatic organisms

B.9.4.1 Summary of studies on toxicity to aquatic organisms

Table B.9.4.1-1 Toxicity of of technical and formulated daminozide and its metabolite to aquatic organisms

Test organism	Test substance	Time scale, study type	Endpoint	Toxicity (mg a.s./L)	Reference
FISH					
Rainbow trout (<i>Oncorhynchus mykiss</i>) ^{# 1}	Daminozide	Acute, 96h (static-renewal)	Mortality, LC ₅₀	n.a.	██████ (1987); FAL 0020
Rainbow trout (<i>Oncorhynchus mykiss</i>) ^{# 1}	Daminozide	Acute, 96h (static)	Mortality, LC ₅₀	n.a.	██████ (1977); A.7.4.1.5
Bluegill sunfish (<i>Lepomis macrochirus</i>) ^{# 1}	Daminozide	Acute, 96h (static)	Mortality, LC ₅₀	n.a.	██████ (1972); A.7.4.1.4
Rainbow trout (<i>Oncorhynchus mykiss</i>) ^{# 1,2}	Alar 85	Acute, 96h (static)	Mortality, LC ₅₀	n.a.	██████ (1966b); A.7.4.1.1
Bluegill sunfish (<i>Lepomis macrochirus</i>) ^{# 1,2}	Alar 85	Acute, 96h (static)	Mortality, LC ₅₀	n.a.	██████ (1966c); A.7.4.1.2
Common carp (<i>Cyprinus carpio</i>)	Dazide Enhance SG	Acute, 96h (semi-static)	Mortality, LC ₅₀	420 form. 357 a.s. (nom)	██████ (2009); 0673/0006
Common carp (<i>Cyprinus carpio</i>)	Dazide Enhance	Acute, 96h (semi-static)	Mortality, LC ₅₀	75 form. 64 a.s. (nom)	██████ (2010); 41004365

Test organism	Test substance	Time scale, study type	Endpoint	Toxicity (mg a.s./L)	Reference
Fathead minnow (<i>Pimephales promelas</i>)	Daminozide	Chronic, 33d ELS (flow-through)	Development and growth, NOEC	1.7 (mm)	(2014); 616A-123
Rainbow trout (<i>Oncorhynchus mykiss</i>) ¹	Methanol	Acute, 96h (flow-through)	Mortality, LC ₅₀	n.a.	Poirier et al. (1986); published literature
Fathead minnow (<i>Pimephales promelas</i>) ¹	Methanol	Acute, 96h (flow-through)	Mortality, LC ₅₀	n.a.	
Bluegill sunfish (<i>Lepomis macrochirus</i>) ¹	Methanol	Acute, 96h (flow-through)	Mortality, LC ₅₀	n.a.	
AQUATIC INVERTEBRATES					
<i>Daphnia magna</i> #	Daminozide	Acute, 96h (flow-through)	Immobility, EC ₅₀	75.5 (mm)	Lintott (1992); A.7.4.1.8
<i>Daphnia magna</i> # ¹	Daminozide	Acute, 48h (static)	Immobility, EC ₅₀	n.a.	Leblanc (1976); A.7.4.1.3
<i>Daphnia magna</i> # ¹	Daminozide	Acute, 48h (static)	Immobility, EC ₅₀	n.a.	Abram (1987); FAL 3
<i>Daphnia magna</i>	Dazide Enhance	Acute, 48h (static)	Immobility, EC ₅₀	60 form. 51 a.s. (nom)	Goodband & Mullee (2010); 41004366
<i>Daphnia magna</i>	Dazide Enhance SG	Acute, 48h (static)	Immobility, EC ₅₀	>100 form.* >85 a.s.* (nom)	Hernádi (2007); 07/482-023DA
<i>Daphnia magna</i> ^{a 1}	Daminozide	Chronic, 21d (semi-static)	Reproduction and development, NOEC	n.a.	Last (2011); 8252736
<i>Daphnia magna</i> ¹	Methanol	Acute, 96h (flow-through)	Immobility, EC ₅₀	n.a.	Dom et al. (2012); published literature
ALGAE					
Freshwater green (<i>Chlorella vulgaris</i>) # ¹	Daminozide	72 h (static)	Growth rate: E _r C ₅₀ Biomass: E _b C ₅₀ NOEC	n.a.	Douglas & Pell (1986); A.7.4.1.7
Freshwater green (<i>Chlorella vulgaris</i>) # ¹	Daminozide	6 d (static)	Growth rate: E _r C ₅₀ Biomass: E _b C ₅₀ NOEC	n.a.	Abram (1987); FAL 4
Freshwater green (<i>Pseudokirchneriella subcapitata</i>)	Daminozide	72 h (static)	Growth rate: E _r C ₅₀ Biomass: E _b C ₅₀ NOEC	>100* >100* 100* (nom)	Manson & Scholey (2006); 2242/049-D2149
Freshwater cyanobacteria (<i>Anabaena flos-aquae</i>)	Daminozide	72 h (static)	Growth rate: E _r C ₅₀ Yield: E _y C ₅₀ NOEC	>100* >100* 100* (nom)	Seeland-Fremer & Mosch (2014); 87711210
Freshwater green (<i>Pseudokirchneriella subcapitata</i>)	Dazide Enhance SG	72 h (static)	Growth rate: E _r C ₅₀	>100 form.* >85 a.s.* >100 form.*	Hernádi (2007); 07/482-022AL

Test organism	Test substance	Time scale, study type	Endpoint	Toxicity (mg a.s./L)	Reference
			Biomass: E _b C ₅₀ Yield: E _y C ₅₀ NOEC	>85 a.s.* >100 form.* >85 a.s.* 100 form.* 85 a.s.* (nom)	
Freshwater green (<i>Pseudokirchneriella subcapitata</i>) ¹	Methanol	96 h (static)	Growth rate: E _r C ₅₀ Yield: E _y C ₅₀ NOEC	n.a.	Cho et al. (2008); published literature
AQUATIC PLANTS					
Duckweed (<i>Lemna gibba</i>) ^{## 1}	Daminozide	7 d (static)	Frond number: EC ₅₀ Dry weight: EC ₅₀ NOEC	n.a.	Palmer <i>et al.</i> (2001); 117A-119
Potential endocrine disrupting properties (Annex Part A, point 8.2.3)					
-					
<p># Study evaluated in old DAR (1999).</p> <p>## Study evaluated in old Addendum 1 (2002).</p> <p>* The highest concentration tested.</p> <p>¹ The study is not considered valid or suitable for regulatory use.</p> <p>² Study summarized and evaluated in Volume 3 CP B.9 for Alar</p> <p>^a Daminozide was tested simultaneously with formaldehyde.</p> <p>(_{nom}) nominal concentration; (_{mm}) mean measured concentration; form.: formulation; a.s.: active substance n.a. not applicable</p>					

No valid chronic study on *Daphnia* and aquatic macrophyte with daminozide is available. In addition, no valid study on aquatic organisms with methanol is available.

Regarding risk to aquatic organisms from methanol, further information was provided by the Notifier (Plath & Kratz, 2017) and it is summarized below:

Notifier's proposal:

Background

*It is noted that the toxicity studies with the metabolite methanol on fish (Poirier et al. 1986, CA 8.2.1/04), *Daphnia magna* (Dom et al. 2012, CA 8.2.4.1/04) and algae (Cho et al. 2008, p. 69, CA 8.2.6.1/03) were rejected by the RMS, and therefore, due to the missing endpoint, the risk assessment was conducted based on the assumption that the metabolite is 10 times more toxic than the parent compound.*

Further consideration

The Notifier understands the arguments leading to the exclusion of the toxicity studies on the metabolite. However, a closer examination of the properties of the metabolite as well as the EFSA Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters (EFSA Journal 2013;11(7):3290) revealed that a risk assessment for methanol is not required.

According to the EFSA Aquatic Guidance document, a metabolite is considered to be not ecotoxicologically relevant and therefore of low risk to the environment "if the metabolite is CO₂ or an inorganic compound that is not a heavy metal—or, it is an organic compound of aliphatic structure, with a chain length of four or less, which consists only of C, H, N or O atoms and which has no 'alerting structures' such as epoxide, nitrosamine, nitrile or other functional groups of known toxicological concern – then no further studies are required [...]". Consequently, methanol (chemical formula: CH₃OH) can be categorized as being of low risk to the environment. These findings are supported when comparing the active substance daminozide and its metabolite methanol using the Q(SAR) model approach based on ECOSAR (Ecological Structure Activity Relationships, US EPA, v1.11). According to ECOSAR, two relevant chemical classes of acid moiety were found that gives the toxic property,

namely hydrazines-acid and amides-acid. These toxophores get lost following the transformation to methanol. Moreover, the aquatic ecotoxicity values predicted by ECOSAR for methanol, based on available linear correlations between toxicity and hydrophobicity, indicate a similar or substantial lower sensitivity of the individual aquatic organism groups to methanol compared to the endpoints for daminozide found in the corresponding submitted studies. Similar findings are observed when including the toxicity values provided by the ECHA database.

These findings support the assumption that methanol does not contain a toxophore moiety. According to the EFSA Aquatic Guidance Document (EFSA Journal 2013;11(7):3290) “as a pragmatic and conservative approach for metabolites without the toxophore, the estimates of exposure could be compared with the RACparent based on the most sensitive endpoint of the a.s. in the relevant compartment. In general, only if this trigger is failed does the toxicity need to be further addressed”. The Risk assessment on aquatic organisms provided by the RMS revealed an acceptable risk for both the outdoor use with an application rate of 4.25 kg a.s./ha (i.e. ornamental plants < 50 cm and > 50 cm) as well as for glasshouse/indoor use with an application rate of 7.565 kg a.s./ha, even when considering an overly conservative chronic endpoint of 1.7 mg/L for *Pimephales promelas* as most sensitive species.

Therefore it can be concluded that additional studies for aquatic organisms with methanol are not required.

Comparison of endpoints for methanol based on the ECHA database and ECOSAR-modelled values in comparison to study-based measured endpoints for daminozide

Organism group	ECHA endpoints for methanol (mg/L) ^a	Predicted endpoints value for Methanol based on ECOSAR (mg/L)	Measured endpoints for daminozide (mg/L)	Reference of measured endpoints for daminozide
Fish	96-h LC50 = 15400	96-h LC50 = 6086.9	96-h LC50 = 64	██████████ (2010a)
Daphnid	96-h EC50 = 182600	48-h LC50 = 2710.4	48-h LC50 = 51	Goodband & Mullee (2010b)
Green Algae	96-h EC50 = 22000	96-h EC50 = 739.1	96-h EC50 > 100	Manson & Scholey (2006), Seeland-Fremer & Mosch (2014)
Fish	28-d NOEC = 446.7	ChV = 446.7 ^b	ChV = 10 ^b	██████████ (2015)
Daphnid	21-d NOEC = 208	ChV = 134.4 ^b	ChV = 1 ^b	Last (2011) ^c
Green Algae	n.d.	ChV = 112.7 ^b	ChV = 100 ^b	Manson & Scholey (2006), Seeland-Fremer & Mosch (2014)

n.d.: not determinable; note the deviating endpoints compared to the modelled ECOSAR endpoints and the measured study endpoint

^a Source: <https://echa.europa.eu/brief-profile/-/briefprofile/100.000.599>

^b ChV: Chronic Value; defined as the geometric mean of the no observed effect concentration (NOEC) and the lowest observed effect concentration (LOEC), mathematically represented as: $ChV = 10^{(\log(LOEC \times NOEC))/2}$

^c Note that this study was rejected by the RMS due to a mixed formulation including formaldehyde. Comparing the ECOSAR predicted endpoints between daminozide and its metabolite methanol and assuming a ten times higher toxicity of methanol compared to daminozide, a 1350 times higher toxicity to Daphnids from methanol compared to daminozide would have to be found based on a formulation without formaldehyde. In consideration of the low formaldehyde content of 16% used in the study by Last (2011), a comparable toxicity of formaldehyde in the formulation used is unlikely; therefore, the use of this measured chronic endpoint for aquatic invertebrates for the comparative approach appears applicable.

Reference list

Cho, C-W., Heon, Y-C., Pham, T.P.T., Vijayaraghavan, K., Yun, Y-S. (2008) The ecotoxicity of ionic liquids and traditional organic solvents on microalga *Selenastrum capricornutum*. *Ecotoxicology and Environmental Safety* Vol. 71, pp. 166-171

Dom, N., Pennick, M., Knapen, D., Blust, R. (2012) Discrepancies in the acute versus chronic toxicity of compounds with a designated narcotic mechanism. *Chemosphere* Vol. 87, pp. 742-749

██████████ (2010a): Acute Toxicity to Common Carp (*Cyprinus carpio*). ██████████

██████████, Report No. 41004365

Goodband, T.J., Mullee, D.M. (2010b): FAL 2400: Acute Toxicity to *Daphnia magna*. Harlan Laboratories Ltd, Report No. 41004366

Last (2012) *Chronic effects to Daphnia magna from exposure to daminozide and formaldehyde*. Covance Laboratories Ltd., Report No. 8252736

Manson, P.S., Scholey, A. (2006) *Daminozide Technical: Inhibition of growth to the alga Pseudokirchneriella subcapitata*. Covance Laboratories Limited, Report No. 2242/049-D2149

██████████ (2015) *Daminozide: An early life-stage toxicity test with the fathead minnow (Pimephales promelas)*. ██████████, Report No. 616A-123

Poirier, S.H., Knuth, M.L., Anderson-Buchou, C.D., Brooke, L.T., Lima, A.R., Shubat, P.J. (1986): *Comparative toxicity of methanol and N,N-dimethylformamide to freshwater fish and invertebrates*. *Bulletin of Environmental Contamination and Toxicology* 37: 615-621

Seeland-Fremer, A., Mosch W. (2014) *Toxicity of daminozide technical to Anabaena flos-aquae in an algal growth inhibition test*. IBACON GmbH, Report No. 87711210

RMS comment on Notifier's proposal:

RMS is of the opinion that studies background data on Q(SAR) modelling should be provided.

This issue should be discussed in peer-review.

B.9.4.2 Risk assessment

The risk assessment is based on the current Guidance Document on Aquatic Ecotoxicology, SANCO/3268/2001, rev 4 final, 17 October 2002. Taking into consideration the EFSA Technical Report 2015 (Outcome of the pesticides peer review meeting on general recurring issues in ecotoxicology), the ErC_{50} values derived from algal toxicity studies were used in the risk assessment.

B.9.4.2.1 Endpoints used in risk assessment

Table B.9.4.2-1 Endpoints of technical and formulated daminozide and its metabolite used in risk assessment

Test organism	Test substance	Time scale, study type	Endpoint	Toxicity (mg a.s./L)	Reference
FISH					
Common carp (<i>Cyprinus carpio</i>)	Dazide Enhance SG	Acute, 96h (semi-static)	Mortality, LC_{50}	420 form. 357 a.s. (nom)	██████████ (2009); 0673/0006
Common carp (<i>Cyprinus carpio</i>)	Dazide Enhance	Acute, 96h (semi-static)	Mortality, LC_{50}	75 form. 64 a.s. (nom)	██████████ (2010); 41004365
Fathead minnow (<i>Pimephales promelas</i>)	Daminozide	Chronic, 33d ELS (flow-through)	Development and growth, NOEC	1.7 (mm)	██████████ (2014); 616A-123
AQUATIC INVERTEBRATES					
<i>Daphnia magna</i>	Daminozide	Acute, 96h (flow-through)	Immobility, EC_{50}	75.5 (mm)	Lintott (1992); A.7.4.1.8
<i>Daphnia magna</i>	Dazide Enhance	Acute, 48h (static)	Immobility, EC_{50}	60 form. 51 a.s. (nom)	Goodband & Mullee (2010); 41004366

Test organism	Test substance	Time scale, study type	Endpoint	Toxicity (mg a.s./L)	Reference
<i>Daphnia magna</i>	Dazide Enhance SG	Acute, 48h (static)	Immobility, EC ₅₀	>100 form. >85 a.s. (nom)	Hernádi (2007); 07/482-023DA
ALGAE					
Freshwater green (<i>Pseudokirchneriella subcapitata</i>)	Daminozide	72 h (static)	Growth rate: E _r C ₅₀	>100 (nom)	Manson & Scholey (2006); 2242/049-D2149
Freshwater cyanobacteria (<i>Anabaena flos-aquae</i>)	Daminozide	72 h (static)	Growth rate: E _r C ₅₀	>100 (nom)	Seeland-Fremer & Mosch (2014); 87711210
Freshwater green (<i>Pseudokirchneriella subcapitata</i>)	Dazide Enhance SG	72 h (static)	Growth rate: E _r C ₅₀	>100 form. >85 a.s. (nom)	Hernádi (2007); 07/482-022AL
AQUATIC PLANTS					
-					
Potential endocrine disrupting properties (Annex Part A, point 8.2.3)					
-					
(nom) nominal concentration; (mm) mean measured concentration; form.: formulation; a.s.: active substance					
n.a. not applicable					

Since no valid chronic toxicity study on *Daphnia* with daminozide was available, no chronic risk assessment for *Daphnia* could be performed. Further, no valid study on aquatic macrophyte was available even if daminozide is a plant growth regulator. Thus, no risk assessment aquatic macrophytes could be performed..

No valid study on aquatic organisms with methanol is available, therefore, the risk assessment for methanol has been performed using toxicity endpoints for daminozide divided by a factor of 10.

B.9.4.2.2 Toxicity exposure ratios for aquatic species for active substance and its metabolites

Aquatic organisms may be exposed to a plant protection product to some extent by spray drift, run-off or drainage from treated fields. The provided studies and data permit a risk assessment following exposure to the product under practical conditions. Predicted environmental concentrations for surface water and sediment (PEC_{sw} and PEC_{sed}) were derived from FOCUS modelling (see Section B.8.) for the proposed use of the formulation Dazide Enhance on ornamentals according to the proposed GAP.

Based on the representative most sensitive endpoint values and the PEC_{sw} values (highest values selected as worst case), the TER-values have been calculated, based on the following equations:

$$TER_a = LC_{50} \text{ or } EC_{50} / \text{initial } PEC_{\text{water}}$$

$$TER_{lt} = E_r C_{50} / \text{initial } PEC_{\text{water}}$$

$$TER_{lt} = \text{chronic NOEC} / \text{long-term } PEC_{\text{water}}$$

The risk is considered acceptable, if the TER_a values for fish and invertebrates are >100, and the TER_{lt} values >10.

B.9.4.2.2.1 Toxicity exposure ratios for aquatic organism based on FOCUSsw Step 1, 2 and 3

In Tables B.9.4.2-2 to B.9.4.2-7, FOCUS PEC_{sw} values for daminozide and its metabolite methanol for the proposed representative use on ornamentals are compared to the results of the standard laboratory aquatic toxicity studies to derive TERs.

Table B.9.4.2-2 Toxicity/exposure ratios for the most sensitive aquatic organisms (Regulation (EU) N° 284/2013, Annex Part A, point 10.2):
FOCUS_{sw} step 1-2 - TERs for daminozide – ornamentals <50 cm (field use) at 4.25 kg a.s./ha x 5

Scenario	PEC _{sw} global max (µg L)	fish acute	fish chronic	Aquatic invertebrates	Aquatic invertebrates prolonged	Algae	Higher plant	Sed. dweller
		<i>Cyprinus carpio</i>	<i>Pimephales promelas</i>	<i>Daphnia magna</i>	-	<i>Pseudokirchneriella subcapitata</i>	-	-
		LC ₅₀	NOEC	EC ₅₀	NOEC	EC ₅₀	EC ₅₀	NOEC
		64000 µg/L	1700 µg/L	51000 µg/L	-	>85000 µg/L	-	-
FOCUS Step 1	1420 µg L	45.07	1.20	35.92	-	>59.86	-	-
FOCUS Step 2								
North Europe	39.09 µg L ^a	1637	43.49	1305	-	-	-	-
South Europe	39.09 µg L ^a	1637	43.49	1305	-	-	-	-
Trigger**		100	10	100	10	10	10	10

Bold figures fall below the Regulation (EU) 546/2011 trigger value

^aPEC_{sw} for a single application as a worse case

^{*}[Only scenarios where the trigger is not met at FOCUS_{sw} step 1-2 should be included in step 3.]

Based on a comparison of the results of the standard laboratory toxicity studies with FOCUS Step 2 maximum PEC_{sw} values for daminozide for ornamentals <50 cm (field use), all TER values were greater than the relevant triggers, indicating low risk.

Table B.9.4.2-3 Toxicity/exposure ratios for the most sensitive aquatic organisms (Regulation (EU) N° 284/2013, Annex Part A, point 10.2):
FOCUS_{sw} step 1-2 - TERs for daminozide – ornamentals >50 cm (field use) at 4.25 kg a.s./ha x 5

Scenario	PEC _{sw} global max (µg L)	fish acute	fish chronic	Aquatic invertebrates	Aquatic invertebrates prolonged	Algae	Higher plant	Sed. dweller
		<i>Cyprinus carpio</i>	<i>Pimephales promelas</i>	<i>Daphnia magna</i>	-	<i>Pseudokirchneriella subcapitata</i>	-	-
		LC ₅₀	NOEC	EC ₅₀	NOEC	EC ₅₀	EC ₅₀	NOEC
		64000 µg/L	1700 µg/L	51000 µg/L	-	>85000 µg/L	-	-
FOCUS Step 1	1500 µg L	42.67	1.13	34.00	-	>56.67	-	-
FOCUS Step 2								
North Europe	113.7 µg L ^a	563	14.95	449	-	-	-	-
South Europe	113.7 µg L ^a	563	14.95	449	-	-	-	-
Trigger**		100	10	100	10	10	10	10

Bold figures fall below the Regulation (EU) 546/2011 trigger value

^aPEC_{sw} for a single application as a worse case

^{*}[Only scenarios where the trigger is not met at FOCUS_{sw} step 1-2 should be included in step 3.]

Based on a comparison of the results of the standard laboratory toxicity studies with FOCUS Step 2 maximum PEC_{sw} values for daminozide for ornamentals >50 cm (field use), all TER values were greater than the relevant triggers, indicating low risk.

**Table B.9.4.2-4 Toxicity/exposure ratios for the most sensitive aquatic organisms (Regulation (EU) N° 284/2013, Annex Part A, point 10.2):
TERs for daminozide – ornamentals (glasshouse/indoor use) at 7.65 kg a.s./ha x 5**

Scenario	PEC _{sw} global max (µg L)	fish acute	fish chronic	Aquatic invertebrates	Aquatic invertebrates prolonged	Algae	Higher plant	Sed. dweller
		<i>Cyprinus carpio</i>	<i>Pimephales promelas</i>	<i>Daphnia magna</i>	-	<i>Pseudokirchneriella subcapitata</i>	-	-
		LC ₅₀	NOEC	EC ₅₀	NOEC	EC ₅₀	EC ₅₀	NOEC
		64000 µg/L	1700 µg/L	51000 µg/L	-	>85000 µg/L	-	-
Glasshouse/indoor	2.562 µg L	24980	664	19906	-	>33177	-	-
Trigger**		100	10	100	10	10	10	10

Bold figures fall below the Regulation (EU) 546/2011 trigger value

^aPEC_{sw} for a single application as a worse case

**[Only scenarios where the trigger is not met at FOCUS_{sw} step 1-2 should be included in step 3.]*

Based on a comparison of the results of the standard laboratory toxicity studies with maximum PEC_{sw} values for daminozide for ornamentals (glasshouse/indoor use), all TER values were greater than the relevant triggers, indicating low risk.

**Table B.9.4.2-5 Toxicity/exposure ratios for the most sensitive aquatic organisms (Regulation (EU) N° 284/2013, Annex Part A, point 10.2):
FOCUS_{sw} step 1-2 - TERs for metabolite methanol – ornamentals <50 cm (field use) at 4.25 kg a.s./ha x 5**

Scenario	PEC _{sw} global max (µg L)	fish acute	fish chronic	Aquatic invertebrates	Aquatic invertebrates prolonged	Algae	Higher plant	Sed. dweller
		<i>Cyprinus carpio</i>	<i>Pimephales promelas</i>	<i>Daphnia magna</i>	-	<i>Pseudokirchneriella subcapitata</i>	-	-
		LC ₅₀	NOEC	EC ₅₀	NOEC	EC ₅₀	EC ₅₀	NOEC
		6400 µg/L ¹	170 µg/L ¹	5100 µg/L ¹	-	>8500 µg/L ¹	-	-
FOCUS Step 1	423.4 µg L	15.12	0.40	12.05	-	>20.08	-	-
FOCUS Step 2								
North Europe	30.34 µg L	211	5.60	168	-	-	-	-
South Europe	35.63 µg L	180	4.77	143	-	-	-	-
Trigger**		100	10	100	10	10	10	10

Bold figures fall below the Regulation (EU) 546/2011 trigger value

¹ There are no valid toxicity data available for the metabolite methanol, therefore it was assumed to be 10 times more toxic than the parent.

*[Only scenarios where the trigger is not met at FOCUS_{sw} step 1-2 should be included in step 3.]

Based on a comparison of the results of the standard laboratory toxicity studies (methanol was assumed to be 10 times more toxic than the parent due to lack of valid toxicity data) with FOCUS Step 1-2 maximum PEC_{sw} values for metabolite methanol for ornamentals <50 cm (field use), all TER values were greater than the relevant triggers, except for chronic fish. Therefore, further consideration is required.

Table B.9.4.2-6 Toxicity/exposure ratios for the most sensitive aquatic organisms (Regulation (EU) N° 284/2013, Annex Part A, point 10.2):
FOCUS_{sw} step 1-2 - TERs for metabolite methanol – ornamentals >50 cm (field use) at 4.25 kg a.s./ha x 5

Scenario	PEC _{sw} global max (µg L)	fish acute	fish chronic	Aquatic invertebrates	Aquatic invertebrates prolonged	Algae	Higher plant	Sed. dweller
		<i>Cyprinus carpio</i>	<i>Pimephales promelas</i>	<i>Daphnia magna</i>	-	<i>Pseudokirchneriella subcapitata</i>	-	-
		LC ₅₀	NOEC	EC ₅₀	NOEC	EC ₅₀	EC ₅₀	NOEC
		6400 µg/L ¹	170 µg/L ¹	5100 µg/L ¹	-	>8500 µg/L ¹	-	-
FOCUS Step 1	497.9 µg L	15.12	0.40	12.05	-	>20.08	-	-
FOCUS Step 2								
North Europe	97.94 µg L	65.35	1.74	52.07	-	-	-	-
South Europe	103.2 µg L	62.02	1.65	49.42	-	-	-	-
Trigger**		100	10	100	10	10	10	10

Bold figures fall below the Regulation (EU) 546/2011 trigger value

¹ There are no valid toxicity data available for the metabolite methanol, therefore it was assumed to be 10 times more toxic than the parent.

*[Only scenarios where the trigger is not met at FOCUS_{sw} step 1-2 should be included in step 3.]

Based on a comparison of the results of the standard laboratory toxicity studies with FOCUS Step 1-2 maximum PEC_{sw} values for metabolite methanol for ornamentals <50 cm (field use), all TER values were below the relevant triggers, except for algae. Therefore, further consideration is required.

**Table B.9.4.2-7 Toxicity/exposure ratios for the most sensitive aquatic organisms (Regulation (EU) N° 284/2013, Annex Part A, point 10.2):
FOCUS_{sw} step 1-2 - TERs for metabolite methanol – ornamentals (glasshouse/indoor use) at 7.65 kg a.s./ha x 5**

Scenario	PEC _{sw} global max (µg L)	fish acute	fish chronic	Aquatic invertebrates	Aquatic invertebrates prolonged	Algae	Higher plant	Sed. dweller
		<i>Cyprinus carpio</i>	<i>Pimephales promelas</i>	<i>Daphnia magna</i>	-	<i>Pseudokirchneriella subcapitata</i>	-	-
		LC ₅₀	NOEC	EC ₅₀	NOEC	EC ₅₀	EC ₅₀	NOEC
		6400 µg/L ¹	170 µg/L ¹	5100 µg/L ¹	-	>8500 µg/L ¹	-	-
Glasshouse/indoor	2.522 µg L	2538	67.41	2022	-	>3370	-	-
Trigger**		100	10	100	10	10	10	10

Bold figures fall below the Regulation (EU) 546/2011 trigger value

¹ There are no valid toxicity data available for the metabolite methanol, therefore it was assumed to be 10 times more toxic than the parent.

*[Only scenarios where the trigger is not met at FOCUS_{sw} step 1-2 should be included in step 3.]

Based on a comparison of the results of the standard laboratory toxicity studies with maximum PEC_{sw} values for methanol for ornamentals (glasshouse/indoor use), all TER values were greater than the relevant triggers, indicating low risk.

Regarding daminozide, it is noted that no valid chronic toxicity data for aquatic invertebrates were available, neither for technical nor for formulated daminozide. No valid aquatic plant toxicity data were available, neither for technical nor for formulated daminozide. Therefore, no risk assessment could be performed for aquatic invertebrates (chronic) and aquatic plants.

In the risk assessment for metabolite methanol, extrapolated endpoints for daminozide were used. Therefore, no risk assessment for aquatic invertebrates (chronic) and aquatic plants could be performed even for methanol.

B.9.4.2.3 Risk to aquatic life from metabolite contamination of groundwater

The possibility of contamination of groundwater from the proposed use of daminozide is evaluated in the EU DAR Volume 3 CP B.8.3. The groundwater exposure assessment was performed for daminozide and its metabolite methanol.

Daminozide, when used according to the EU-representative GAP, will not pose a risk to the groundwater compartment – all calculated PEC_{GW} values for this compound were well below the trigger of 0.1 µg/L (the reported values were <0.001 µg/L for all scenarios). The similar conclusion can be stated for the metabolite methanol – the calculated PEC_{GW} values were <0.1 µg/L for all scenarios.

B.9.4.3 Environmental Hazard Classification/Labelling

Proposal for classification of the active substance for environmental effects according to Regulation (EC) 1272/2008

Pictogram	None
Signal word	None
Classification categories:	None
M-factor (acute/chronic)	None
Hazard statements	None
Precautionary statements	P391 Collect spillage P501 Dispose of contents/ container to ... (in accordance with local/ regional/ national/ international regulation (to be specified))

Justification for the proposal:

Aquatic acute classification: Acute crustacean and algal toxicity data were only available for active substance while no valid acute fish toxicity endpoint was available. Therefore, a surrogate endpoint LC_{50} of 64 mg a.s./L derived from the acute toxicity study with formulation Dazide Enhance on *Cyprinus carpio* was used. This endpoint was the lowest one and based on it, no aquatic acute classification is required for daminozide.

Aquatic chronic classification: Chronic fish and algal toxicity data were available for active substance; the lower endpoint was derived from chronic fish ELS study (*Pimephales promelas*, $NOEC = 1.7$ mg a.s./L). Taking into account that daminozide is rapidly degradable substance, no aquatic chronic classification is required ($NOEC$ is > 1 mg a.s./L).

However, no valid chronic crustacean toxicity data neither for technical nor for formulated daminozide were available. Therefore, acute toxicity data for

crustacea were used as a surrogate system (*Daphnia magna*, EC50 = 75.5 mg a.s./L). Taking into account that daminozide is rapidly degradable substance and has log Kow <4, no aquatic chronic classification is required for daminozide.

Proposal for classification of the formulation Dazide Enhance for environmental effects according to Regulation (EC) 1272/2008

Pictogram	None
Signal word	None
Classification categories:	None
M-factor (acute/chronic)	None
Hazard statements	None
Precautionary statements	P501 Dispose of contents/ container to ... (in accordance with local/ regional/ national/ international regulation (to be specified))

Justification for the proposal:

Aquatic acute classification:	<p>Aquatic acute toxicity data for all three trophic levels were available for formulation Dazide Enhance. The lowest endpoint was LC₅₀ = 60 mg form./L for <i>Daphnia magna</i>:</p> <p>LC₅₀ > 1 mg a.s./L, therefore, <u>no aquatic acute classification is required for formulation Dazide Enhance.</u></p>
Aquatic chronic classification:	<p>The chronic endpoint was available for algae only and not for the other trophic species levels (algal NOEC = 100 mg form./L).</p> <p>Based on chronic fish endpoint for active substance (<i>Pimephales promelas</i>, NOEC = 1.7 mg a.s./L), content 85% of active substance in formulation and the fact that daminozide is rapidly degradable substance, no aquatic chronic classification is required for fish and algae.</p> <p>No valid chronic crustacean toxicity data neither for technical nor for formulated daminozide were available. However, taking into account no need for aquatic chronic classification for active substance, content 85% of active substance in formulation, the fact that daminozide is rapidly degradable substance and has log Kow <4, <u>no aquatic chronic classification is required for formulation Dazide Enhance.</u></p>

Conclusion – risk to aquatic organisms

No acute risks were identified for fish and aquatic invertebrates and no chronic risks were identified for fish and algae from daminozide and its metabolite methanol.

No valid chronic toxicity data for aquatic invertebrates and aquatic macrophytes were available, neither for daminozide nor for methanol. Therefore, no chronic risk assessment could be performed for aquatic invertebrates and aquatic macrophytes. Thus, risk assessment for both daminozide and methanol could not be finalized.

B.9.5 Effects on arthropods

B.9.5.1 Effects on bees

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B.9.5.2 Effects on non-target arthropods other than bees

B.9.5.2.1 Standard laboratory testing for non-target arthropods

i) Effects on *Typhlodromus pyri* in laboratory test

Reference:	Harwood, R. W. J. (2000) A laboratory evaluation of the side effects of daminozide on the predatory mite <i>Typhlodromus pyri</i> .
Report No.:	18133
Guideline:	IOBC/WPRS Overmeer (1988), improvements by Louis & Uffer (1995)
GLP:	No
Previous evaluation:	In Addendum 1 (2002)
Material and methods:	
Test material:	Dazide 85 (85% daminozide)
Lot/Batch No:	Not stated
Purity:	Not stated

A laboratory test to determine the effects of Dazide 85 (85 % a.s.) soluble powder formulation on the predatory mite, *Typhlodromus pyri* was performed. Test was based on methods of Overmeer (1988) and Louis and Ufer (1995). Dazide 85 was applied to glass test arenas (consisting of vinyl tile of approx. 10 x10 cm) at twice its maximum recommended rate (8.5 a.s. kg/ha). The sprayer was calibrated prior to application and treatments were applied to the upper surface of the test arenas and to both sides of the glass cover slips used for shelters. Twenty, 2- 3 days old mites were placed in each test arena and in each treatment and 5 replicates were used. A small shelter made from broken glass cover slips was placed near the centre of test arenas and a small amount of pollen (*Pinus nigra*) was provided for food. Mortality assessments were performed at ca. 24 h after application and again at 7 days after application. Surviving males and females were transferred to clean test boxes to a sex ratio of 4 males to 7 females to unused test arenas 7 days after application. After 7 days of transfer (i.e. 14 days

after application) an assessment of egg production was carried out. A control with water and a toxic reference treatment with Dimethoate (40 EC 0.17 L/ha) were used.

Results:

Mean mortality in the water control was 4% after 24 h. In the Dazide 85 and in the reference toxicant treatment, mean corrected mortality was 67 and 30%, respectively after 24 h. After 7 days of application mortality increased to 19% in the control and 100% in the reference toxicant. In the Dazide 85 control corrected mortality was 98%. For the fecundity assessments, no live females were available in the Dazide 85 treatment after 7 days of application. After 14 days of applications a total of 54 eggs and newly hatched larvae were found in water control. The number of eggs per female was 1.54.

The results of mortality and fecundity assessment are summarized in the table below.

Table B.9.5.2-1 Mortality and fecundity results

Treatment	Mortality after 7 days		Fecundity		
	Mortality	Corrected mortality	Total females transferred	Total eggs laid	No. off eggs per female
Control	19%	-	35	54	1.54
Daminozide, 8.5 kg a.s./ha	98%	98%	0	-	-
Dimethoate 40	100%	-	-	-	-

Remark from previous review: The author states that the effect of Dazide 85 may have due to the sticky nature of the formulation rather than to direct toxic effects caused by contact exposure. The number of eggs per females in the control is too low. The validity criteria for fecundity (i.e. 4 eggs per female) are thus not met. The results are not used for risk assessment.

RMS comments and conclusion:

The non-GLP study was conducted according to the IOBC/WPRS guideline Overmeer (1988). It is also in line with the current guideline Blümel *et al.* (2000).

The study results meet the guidelines' validity criteria (mortality in the control treatment over the initial 7 days should not exceed 20%: actual value was 19%; corrected mortality in the toxic reference treatment should be 50-100%: actual value was 100% at 7 DAT), except for the mean cumulative number of eggs produced between 7 and 14 days (should be greater than 4.0 per female in the control treatment: actual number of eggs per female was 1.54).

Therefore, the study is not considered valid.

B.9.5.2.2 Extended laboratory testing, aged residue studies with non-target arthropods**i) Effects on *Typhlodromus pyri* in extended laboratory test**

Reference:	Taruza, S. (2001b) An extended laboratory test to determine the effect of fresh residues of Dazide 85 on the predatory mite <i>Typhlodromus pyri</i> (Acari; Phytoseiidae)
Report No.:	RIV-02-01
Guideline:	Blümel <i>et al.</i> (2000)
GLP:	Yes
Previous evaluation:	In Addendum 1 (2002)
Material and methods:	
Test material:	Dazide 85 (85% daminozide)
Lot/Batch No:	40/11/00
Purity:	85.3% w/w

Toxicity test with Dazide 85 (85% nominally) on the mite *Typhlodromus pyri* was conducted under extended laboratory conditions. Dazide 85 was tested in three concentrations of 8.824, 4.412 and 1.176 kg product/ha, equivalent to 7.5, 3.75, and 1.0 kg as/ha, in quintuplicate. As a negative control deionised water and as a positive control dimethoate (400 g/L) were included in the experiments. Treatments were applied with a calibrated sprayer to leaf disks of the bean *Phaseolus vulgaris*. Leaf disks were placed in an arena, on top of wet cotton wool. A ring of sticky gel around the leaf prevented the escape of the mites. Twenty protonymphal mites were placed at the centre of the treated leaf disc one hour after treatments were applied. Each treatment was performed with 5 replicates. Mortality was determined at 1 and 7 days after application. Fecundity was determined 7, 10, 13 and 14 days after application. The results of mortality and fecundity were compared by one-way ANOVA.

Results:

Control mortality was 14%. Control corrected mortality in the Dazide 85 treatments was 26, 8 and 5% for 7.5, 3.75 and 1.0 kg/ha, respectively. Corrected mortality in the positive control was 53% (significant at $P < 0.01$). The validity criteria (control mortality $< 20\%$ and mortality in the positive control $> 50\%$) were met. Mean number of eggs produced per female was 8.1 in the control treatment and 6.5, 7.6 and 8.1 in the 7.5, 3.75 and 1.0 kg as/ha treatment rates of Dazide 85, respectively. This was equivalent to changes in egg production of -20, -6 and 0%, relative to the control, in the respective test item treatment rates.

The results of mortality assessment are summarized in the table below.

Table B.9.5.2-2 Mean percentage mortality of mites at 7 DAT

Treatment		Mean % mortality at 7 DAT	Corrected % mortality at 7 DAT
Control		14	-
Dazide 85	7.5 kg a.s./ha	36	26
	3.75 kg a.s./ha	21	8
	1.0 kg a.s./ha	18	5
Perfekthion	6 g a.s./ha	60 **	53

The results were compared by one-way ANOVA and asterisks indicate treatments that differed significantly from the control (** P < 0.01).

The results of fecundity assessment are summarized in the table below.

Table B.9.5.2-3 A summary of the fecundity of mites. The data presented are the mean number of eggs laid per female between 7 and 14 DAT and the mean fecundity in each treatment, relative to control

		Mean number of eggs per female	% change in numbers of eggs produced, relative to the control
Control		8.1	-
Dazide 85	7.5 g a.s./ha	6.5	-20
	3.75 g a.s./ha	7.6	-6
	1.0 g a.s./ha	8.1	0

The results were compared by one-way ANOVA, but treatments did not differ significantly.

Remark from previous review: The result that Dazide 85 was slightly harmful to the mite *Typhlodromus pyri* at a rate of 7.5 kg as/ha was used for the risk assessment. At lower rates of 3.75 and 1.0 kg as/ha Dazide was not harmful.

RMS comments and conclusion:

The reported study is GLP compliant and was conducted according to the IOBC/WPRS guideline Blümel *et al.* (2000). The study results meet the guidelines' validity criteria (mortality in the control treatment over the initial 7 days should not exceed 20%: actual value was 14%; corrected mortality in the toxic reference treatment should be 50-100%: actual value was 53% at 7 DAT; the mean cumulative number of eggs produced between 7 and 14 days should be greater than 4.0 per female in the control treatment: actual number of eggs per female was 8.1). The study is acceptable for regulatory use.

The 7-day LR₅₀ and the 14-day ER₅₀ is >8.824 kg formulation/ha (equivalent to 7.5 kg a.s./ha).

B.9.6 Risk assessment for arthropods

B.9.6.1 Risk assessment for bees

B.9.6.1.1 Summary of bee toxicity studies

Summary of reported laboratory bee toxicity studies carried out with technical and formulated daminozide is given in Table B.9.6.1-1.

Table B.9.6.1-1 Summary of reported laboratory bee toxicity studies with technical and formulated daminozide

Species	Test substance	Time scale/type of endpoint	End point	Toxicity	Reference
Acute oral and contact toxicity (laboratory)					
<i>Apis mellifera</i> [#]	Daminozide	Acute	Oral toxicity (LD ₅₀)	>200 µg a.s./bee	Davies, 1987; FAL 5
<i>Apis mellifera</i> [#]	Daminozide	Acute	Contact toxicity (LD ₅₀)	>200 µg a.s./bee	
<i>Apis mellifera</i> ^{#1}	Alar 85	Acute	Oral toxicity (LD ₅₀)	>100 µg form./bee >85 µg a.s./bee	Cole, 1985; A.7.4.2.7
<i>Apis mellifera</i> ^{#1}	Alar 85	Acute	Contact toxicity (LD ₅₀)	>100 µg form./bee >85 µg a.s./bee	
Chronic toxicity to adult bees (laboratory)					
<i>Apis mellifera</i>	Daminozide	Chronic	10 d chronic toxicity (LDD ₅₀)	>106.2 µg a.is/bee/day	Haupt, 2014; 87715136
Larval toxicity (laboratory)					
<i>Apis mellifera</i>	Daminozide	Chronic, repeated exposure	Oral toxicity (NOED)	100 µg a.s./larva	Odemer, 2015; 20150038

[#] Study evaluated in old DAR (1999).

¹ Study summarized and evaluated in Volume 3 CP B.9 for Alar

B.9.6.1.2 Risk assessment for bees

EFSA Guidance on the risk assessment of plant protection products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees) (EFSA Journal 2013;11(7):3295) was published already in July 2013, but it has not come into force yet. However, based on the Technical report on the outcome of the pesticides peer review meeting on

general recurring issues in ecotoxicology (EFSA, 2015), the risk assessment for bees (first tier) should be carried out according to EFSA Guidance, therefore it has been used in the present risk assessment.

The risk assessment was performed for field use (4.25 kg a.s./ha). For protected use (7.65 kg a.s./ha), the risk assessment for bees should be performed assuming the same exposure as for a field use, unless it is indicated that the uses will be restricted to permanent greenhouses (based on Technical report on the outcome of the pesticides peer review meeting on general recurring issues in ecotoxicology (EFSA, 2015)). This was not indicated in the GAP. Therefore, the risk is considered low for permanent greenhouses and no risk assessment is required for bees, however, for protected use other than permanent glasshouses, the risk assessment for bees assuming the same exposure as for a field use was carried out.

The risk assessment was carried out for daminozide and formulation Dazide Enhance.

It is noted that no scenario for ornamentals is included in the EFSA Guidance (2013). Therefore, a surrogate scenario for leafy vegetables has been used by RMS. However, this should be discussed in peer-review.

Risk assessment for honeybees:

1) Field use

Table B.9.6.1-2 Risk assessment for bees from contact and oral dietary exposure for ornamentals (field use) at 4.25 kg a.s./ha x 5, BBCH <50

Species	Test substance	Scenario	Risk quotient	HQ/ETR	Trigger
Screening level assessment					
<i>Apis mellifera</i>	a.s.	Not relevant	HQ _{contact}	<21.3	42
<i>Apis mellifera</i>	a.s.	Not relevant	ETR _{acute adult oral}	<0.16	0.2
<i>Apis mellifera</i>	Preparation	Not relevant	HQ _{contact}	<90	42
<i>Apis mellifera</i>	Preparation	Not relevant	ETR _{acute adult oral}	<0.68	0.2
<i>Apis mellifera</i>	a.s.	Not relevant	ETR _{chronic adult oral}	<0.304	0.03
<i>Apis mellifera</i>	a.s.	Not relevant	ETR _{chronic larva oral}	0.19	0.2
Tier 1 level assessment – BBCH <10 (leafy vegetables)					
<i>Apis mellifera</i>	a.s.	treated crop	ETR _{chronic adult oral}	0.016	0.03
<i>Apis mellifera</i>	a.s.	weeds	ETR _{chronic adult oral}	0.084	0.03
<i>Apis mellifera</i>	a.s.	field margin	ETR _{chronic adult oral}	0.001	0.03
<i>Apis mellifera</i>	a.s.	adjacent crop	ETR _{chronic adult oral}	0.001	0.03
<i>Apis mellifera</i>	a.s.	succeeding crop	ETR _{chronic adult oral}	0.016	0.03
Tier 1 level assessment – BBCH 10-49 (leafy vegetables)					
<i>Apis mellifera</i>	a.s.	treated crop	ETR _{chronic adult oral}	0.167	0.03
<i>Apis mellifera</i>	a.s.	weeds	ETR _{chronic adult oral}	0.084	0.03
<i>Apis mellifera</i>	a.s.	field margin	ETR _{chronic adult oral}	0.001	0.03
<i>Apis mellifera</i>	a.s.	adjacent crop	ETR _{chronic adult oral}	0.001	0.03
<i>Apis mellifera</i>	a.s.	succeeding crop	ETR _{chronic adult oral}	0.016	0.03

Figures in bold exceed the relevant trigger value

Table B.9.6.1-3 Risk assessment for honeybees from consumption of contaminated water

Species	Test substance	Risk quotient	ETR	Trigger
Risk assessment from exposure to residues in guttation fluid (water solubility = 128 g/L)				
<i>Apis mellifera</i>	a.s.	ETR _{acute adult oral}	7.3	0.2
<i>Apis mellifera</i>	a.s.	ETR _{chronic adult oral}	7.42	0.03
<i>Apis mellifera</i>	a.s.	ETR _{chronic larva oral}	102.3	0.2
Risk assessment from exposure to residues in surface water (FOCUS step 2 PEC _{sw} of 0.1 mg/L)				
<i>Apis mellifera</i>	a.s.	ETR _{acute adult oral}	0.00	0.2
<i>Apis mellifera</i>	a.s.	ETR _{chronic adult oral}	0.000	0.03
<i>Apis mellifera</i>	a.s.	ETR _{chronic larva oral}	0.00	0.2
Risk assessment from exposure to residues in puddle water				
<i>Apis mellifera</i>	a.s.	ETR _{acute adult oral}	0.00	0.2
<i>Apis mellifera</i>	a.s.	ETR _{chronic adult oral}	0.000	0.03
<i>Apis mellifera</i>	a.s.	ETR _{chronic larva oral}	0.00	0.2

Figures in bold exceed the relevant trigger value

Both acute adult HQ_{contact} and ETR_{acute adult oral} values for formulation did not meet the relevant triggers at screening assessment. However, the acute oral and contact LD₅₀ values were derived from the limit test carried out with 100 µg formulation./bee (equivalent to 85 µg a.s./bee). Corrected mortality after 48 hours was reported to be about 23% for oral and about 20% for contact exposure. Since calculated HQ_{contact} and ETR_{acute adult oral} for formulation are rather close to the relevant triggers and real LD₅₀ is supposed to be much higher than 100 µg formulation./bee, it is considered acceptable to base the risk assessment on active substance toxicity data only.

All the HQ and ETR values for active substance met the relevant triggers at screening assessment, except for the chronic oral risk to adult honeybees. Therefore, Tier 1 assessment was performed for chronic oral risk to adult honeybees. All the ETR values for active substance met the relevant triggers at Tier 1 assessment, except for scenario “treated crop” at BBCH 10-49 and scenario “weeds” at all BBCH considered.

Regarding the chronic adult risk for “treated crop” scenario, the Notifier provided the following justification: “Considering that Dazide Enhance is a plant growth regulator that interferes with gibberellic acid biosynthesis to cause the plant to grow more “compacted” (by inhibition of intermodal elongation) and is applied by knapsack sprayer prior to flowering, the crop will not be attractive to foraging bees. ... Daminozide is also not persistent in soil (maximum DT₅₀ of 0.37 days) so residues are not expected to be taken up by plants at significant levels later in the growing season when flowers are present.” This is agreed by the RMS and the chronic risk to bees from the proposed use of Dazide Enhance is considered to be low.

Regarding the chronic adult risk for “weeds” scenario, the Notifier provided the following risk assessment:

First tier assessment for oral route of exposure – foraging on weeds in the treated field

Test group	Exposure scenario	Appln. rate (kg a.s./ha)	Ef	Short-cut value	twa	Endpoint	ETR _{oral}	Trigger	Acceptable risk?
Weeds in the field									
Honey bee (adults)	Chronic oral	4.25	0.4 ^a	2.9 µg ^b	0.72	> 106.2 µg/bee	< 0.033	0.03	Yes
			0.4 ^a	0.27 µg ^c	0.72		< 0.003	0.03	Yes

^a As application is until BBCH 50 and no default value is available for ornamentals BBCH <50, a deposition factor of 60% is assumed, for plants with a similar structure (e.g. strawberries)

^b Application after emergence of weeds

^c Application before emergence of weed

Ef: exposure factor

twa: time weighted average (default)

RMS: It is noted that according to EFSA GD (2013) deposition factor of 0.3 should be used for ornamentals (surrogate value from leafy vegetables). Anyway, the calculation of ETR_{chronic adult oral} performed by the Notifier are not in accordance with the calculation done by RMS.

In case of unacceptable chronic adult risk to honeybees for “weeds” scenario, the risk could be mitigated by applying when flowering weeds are not present in crop.

Regarding the risk assessment for honeybees from consumption of contaminated water, all the ETR values for active substance met the relevant triggers, except for exposure to residues in guttation fluid. No refinement was available.

2) Protected use

Table B.9.6.1-4 Risk assessment for bees from contact and oral dietary exposure for ornamentals (protected use) at 7.65 kg a.s./ha x 5, BBCH <50

Species	Test substance	Scenario	Risk quotient	HQ/ETR	Trigger
Screening level assessment					
<i>Apis mellifera</i>	a.s.	Not relevant	HQ _{contact}	<38.3	42
<i>Apis mellifera</i>	a.s.	Not relevant	ETR _{acute adult oral}	<0.29	0.2
<i>Apis mellifera</i>	Preparation	Not relevant	HQ _{contact}	<50	42
<i>Apis mellifera</i>	Preparation	Not relevant	ETR _{acute adult oral}	<0.38	0.2
<i>Apis mellifera</i>	a.s.	Not relevant	ETR _{chronic adult oral}	<0.547	0.03
<i>Apis mellifera</i>	a.s.	Not relevant	ETR _{chronic larva oral}	0.34	0.2

Tier 1 level assessment – BBCH <10 (leafy vegetables)					
<i>Apis mellifera</i>	a.s.	treated crop	ETR _{acute adult oral}	0.03	0.2
<i>Apis mellifera</i>	a.s.	treated crop	ETR _{chronic adult oral}	0.028	0.03
<i>Apis mellifera</i>	a.s.	treated crop	ETR _{acute larva oral}	0.03	0.2
<i>Apis mellifera</i>	a.s.	weeds	ETR _{acute adult oral}	0.14	0.2
<i>Apis mellifera</i>	a.s.	weeds	ETR _{chronic adult oral}	0.150	0.03
<i>Apis mellifera</i>	a.s.	weeds	ETR _{acute larva oral}	0.14	0.2
<i>Apis mellifera</i>	a.s.	field margin	ETR _{acute adult oral}	0.00	0.2
<i>Apis mellifera</i>	a.s.	field margin	ETR _{chronic adult oral}	0.001	0.03
<i>Apis mellifera</i>	a.s.	field margin	ETR _{acute larva oral}	0.00	0.2
<i>Apis mellifera</i>	a.s.	adjacent crop	ETR _{acute adult oral}	0.00	0.2
<i>Apis mellifera</i>	a.s.	adjacent crop	ETR _{chronic adult oral}	0.001	0.03
<i>Apis mellifera</i>	a.s.	adjacent crop	ETR _{acute larva oral}	0.00	0.2
<i>Apis mellifera</i>	a.s.	succeeding crop	ETR _{acute adult oral}	0.03	0.2
<i>Apis mellifera</i>	a.s.	succeeding crop	ETR _{chronic adult oral}	0.028	0.03
<i>Apis mellifera</i>	a.s.	succeeding crop	ETR _{chronic larva oral}	0.03	0.2
Tier 1 level assessment – BBCH 10-49 (leafy vegetables)					
<i>Apis mellifera</i>	a.s.	treated crop	ETR _{acute adult oral}	0.29	0.2
<i>Apis mellifera</i>	a.s.	treated crop	ETR _{chronic adult oral}	0.301	0.03
<i>Apis mellifera</i>	a.s.	treated crop	ETR _{acute larva oral}	0.29	0.2
<i>Apis mellifera</i>	a.s.	weeds	ETR _{acute adult oral}	0.14	0.2
<i>Apis mellifera</i>	a.s.	weeds	ETR _{chronic adult oral}	0.150	0.03
<i>Apis mellifera</i>	a.s.	weeds	ETR _{acute larva oral}	0.14	0.2
<i>Apis mellifera</i>	a.s.	field margin	ETR _{acute adult oral}	0.00	0.2
<i>Apis mellifera</i>	a.s.	field margin	ETR _{chronic adult oral}	0.001	0.03
<i>Apis mellifera</i>	a.s.	field margin	ETR _{acute larva oral}	0.00	0.2
<i>Apis mellifera</i>	a.s.	adjacent crop	ETR _{acute adult oral}	0.00	0.2
<i>Apis mellifera</i>	a.s.	adjacent crop	ETR _{chronic adult oral}	0.001	0.03
<i>Apis mellifera</i>	a.s.	adjacent crop	ETR _{acute larva oral}	0.00	0.2
<i>Apis mellifera</i>	a.s.	succeeding crop	ETR _{acute adult oral}	0.03	0.2
<i>Apis mellifera</i>	a.s.	succeeding crop	ETR _{chronic adult oral}	0.028	0.03
<i>Apis mellifera</i>	a.s.	succeeding crop	ETR _{chronic larva oral}	0.03	0.2

Figures in bold exceed the relevant trigger value

Table B.9.6.1-5 Risk assessment for honeybees from consumption of contaminated water

Species	Test substance	Risk quotient	ETR	Trigger
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Risk assessment from exposure to residues in guttation fluid (water solubility = 128 g/L)

Species	Test substance	Risk quotient	ETR	Trigger
<i>Apis mellifera</i>	a.s.	ETR _{acute adult oral}	7.3	0.2
<i>Apis mellifera</i>	a.s.	ETR _{chronic adult oral}	7.42	0.03
<i>Apis mellifera</i>	a.s.	ETR _{chronic larva oral}	102.3	0.2
Risk assessment from exposure to residues in surface water (FOCUS step 2 PEC _{sw} of 0.1 mg/L)				
<i>Apis mellifera</i>	a.s.	ETR _{acute adult oral}	0.00	0.2
<i>Apis mellifera</i>	a.s.	ETR _{chronic adult oral}	0.000	0.03
<i>Apis mellifera</i>	a.s.	ETR _{chronic larva oral}	0.00	0.2
Risk assessment from exposure to residues in puddle water				
<i>Apis mellifera</i>	a.s.	ETR _{acute adult oral}	0.00	0.2
<i>Apis mellifera</i>	a.s.	ETR _{chronic adult oral}	0.000	0.03
<i>Apis mellifera</i>	a.s.	ETR _{chronic larva oral}	0.00	0.2

Figures in bold exceed the relevant trigger value

No HQ or ETR values for active substance met the relevant triggers at screening assessment, except for the acute contact risk to adult honeybees. Therefore, Tier 1 assessment was performed for acute oral and chronic oral risk to adult honeybees and for acute oral risk to larvae. All the ETR values for active substance met the relevant triggers at Tier 1 assessment, except for scenario “treated crop” at BBCH 10-49 and for chronic oral risk to adult honeybees for scenario “weeds” at all BBCH considered.

For permanent greenhouses, the exposure will be negligible and the risk to honeybees is considered low. However, for the other protected uses, acute and chronic oral risk to adult honeybees and acute oral risk to larvae was identified as high.

It is noted that the proposed GAP for daminozide includes ornamentals at BBCH <50 (i.e. prior to flowering), therefore, the crop will not be attractive for honeybees foraging on pollen and nectar. As regards to unacceptable chronic adult risk to honeybees for “weeds” scenario, the risk could be mitigated by applying when flowering weeds are not present in crop.

Risk assessment for bumblebees and solitary bees:

No data were available and no risk assessment was performed by RMS.

Since a risk to pollinators introduced in glasshouses where daminozide is used could not be excluded, risk mitigation measures such as covering or removing bumble bee colonies for the application are proposed for these situations.

Conclusion – risk to bees

No risks were identified for bees for field use and protected use (other than permanent greenhouses) when relevant mitigation measures are considered, except for consumption of guttation fluid where high risk was concluded.

No risks were identified for bees for protected use in permanent greenhouses when relevant mitigation measures are considered.

The risk assessment for bees should be discussed in peer-review.

B.9.6.2 Risk assessment for non-target arthropods other than bees

B.9.6.2.1 Summary of toxicity to non-target arthropods other than bees

Studies on toxicity to non-target arthropods are summarized in the following tables:

Table B.9.6.2-1 Laboratory tests with non-target arthropods

Species	Life stage	Test substance	Study type	Dose (kg /ha) ²	Mortality/ Corr. mortality (%)	Sublethal effects ³	References
Laboratory tests							
<i>Aphidius rhopalosiphi</i>	Adult ⁴	Alar 85 SP	Tier I Glass plate Limit test	Control 10 form. (8.5 a.s.)	2.5 12.5 / 10 LR ₅₀ >10 kg form./ha (>8.5 kg a.s./ha)	No. of pupae / % adverse effects 21.1 22.6 / -7.1% ER ₅₀ >10 kg form./ha (>8.5 kg a.s./ha)	Baxter 1999a; UNI-99-9
<i>Typhlodromus pyri</i>	Protonymph ¹	Daminozide	Tier I Glass plate Limit test	Control 7.225 a.s.	n.a.	n.a.	Harwood 2000; 18099
	Protonymph ¹	Dazide 85	Tier I Glass plate Limit test	Control 10 form. (8.5 a.s.)	n.a.	n.a.	Harwood 2000; 18133
	Protonymph ⁴	Alar 85 SP	Tier I Glass plate Limit test	Control 10 form. (8.5 a.s.)	3 14 / 11.3 LR ₅₀ >10 kg form./ha (>8.5 kg a.s./ha)	No. of eggs per female / % adverse effects 7.2 3.9 / 45.8% ER ₅₀ >10 kg form./ha (>8.5 kg a.s./ha)	Vinall 1999; UNI-99-8

Species	Life stage	Test substance	Study type	Dose (kg /ha) ²	Mortality/ Corr. mortality (%)	Sublethal effects ³	References
<i>Encarsia formosa</i>	Adult ⁴	Alar 85 SP	Tier I Glass plate Limit test	Control 10 form. (8.5 a.s.)	18 85 / 82 LR ₅₀ <10 kg form./ha (<8.5 kg a.s./ha)	No. of parasitized scales / % adverse effects 18.2 17.8/ 2.2% ER ₅₀ >10 kg form./ha (>8.5 kg a.s./ha)	Halsall 2000; UNI-00-2
<i>Orius laevigatus</i>	Adult ⁴	Alar 85 SP	Tier I Glass plate Limit test	Control 10 form. (8.5 a.s.)	17 14 / 0 LR ₅₀ >10 kg form./ha (>8.5 kg a.s./ha)	No. of eggs per female / % adverse effects 7.5 7.9 / -5.3% ER ₅₀ >10 kg form./ha (>8.5 kg a.s./ha)	Vinall 2000; UNI-00-3
<i>Poecilus cupreus</i>	Adult ⁴	Alar 85 SP	Tier I Glass plate Limit test	Control 10 form. (8.5 a.s.)	0 0 / 0 LR ₅₀ >10 kg form./ha (>8.5 kg a.s./ha)	No. of larvae consumed / % adverse effects 4.83 4.90 / -1.4% ER ₅₀ >10 kg form./ha (>8.5 kg a.s./ha)	Baxter 1999b; UNI-99-10
<i>Chrysoperla carnea</i>	Larva ⁴	Alar 85 SP	Tier I Glass plate Limit test	Control 10 form. (8.5 a.s.)	10 12 / 2 LR ₅₀ >10 kg form./ha (>8.5 kg a.s./ha)	No. of eggs per female / % adverse effects 15.7 15.4 / 1.9 ER ₅₀ >10 kg form./ha (>8.5 kg a.s./ha)	Barton 1999; UNI-99-11
Extended laboratory tests							

Species	Life stage	Test substance	Study type	Dose (kg /ha) ²	Mortality/ Corr. mortality (%)	Sublethal effects ³	References
<i>Typhlodromus pyri</i>	Protonymph ⁴	Alar 85 SP	Tier I Glass plate	Control 5 form. (4.25 a.s.) 10 form. (8.5 a.s.)	19 23 / 5 15 / 0 LR ₅₀ >10 kg form./ha (>8.5 kg a.s./ha)	No. of eggs per female / % adverse effects 5.3 6.0 / -13.2% 5.1 / 3.8% ER ₅₀ >10 kg form./ha (>8.5 kg a.s./ha)	Taruza 2001a; UNI-01-1
<i>Typhlodromus pyri</i>	Protonymph	Dazide 85	Tier I Glass plate	Control 1.176 form. (1.0 a.s.) 4.412 form. (3.75 a.s.) 8.824 form. (7.5 a.s.)	14 18 / 5 21 / 8 36 / 26 LR ₅₀ >8.824 kg form./ha (>7.5 kg a.s./ha)	No. of eggs per female / % adverse effects 8.1 8.1 / 0% 7.6 / 6.2% 6.5 / 19.8% ER ₅₀ >8.824 kg form./ha (>7.5 kg a.s./ha)	Taruza 2001b; RIV-02-1

¹ the study is not considered valid

² form. – formulation; a.s. - active substance

³ positive percentages relate to adverse effects in comparison with control

⁴ Study summarized and evaluated in Volume 3 CP B.9 for Alar

n.a. – not applicable

It is noted that two formulations were tested: Alar 85 SP and Dazide 85. They are earlier formulations of Alar and Dazide Enhance, respectively, and their toxicities are considered to be comparable with the toxicity of the current formulation Dazide Enhance. Therefore, endpoints derived from all the studies on non-target arthropods can be used for the risk assessment for Dazide Enhance.

B.9.6.2.2 Risk assessment for non-target arthropods other than bees

The product Dazide Enhance is intended to be used as a foliar spray on ornamentals, with an application rate of 4.25 kg daminozide/ha for field use and 7.65 kg daminozide/ha for glasshouse use, in a maximum of 5 application per year, at a minimum interval of 7 days.

The risk assessment was performed for field use (4.25 kg a.s./ha). For protected use (7.65 kg a.s./ha), the risk assessment for non-target arthropods should be performed assuming the same exposure as for a field use, unless it is indicated that the uses will be restricted to permanent greenhouses (based on Technical report on the outcome of the pesticides peer review meeting on general recurring issues in ecotoxicology (EFSA, 2015)). This

was not indicated in the GAP. Therefore, the risk is considered low for permanent greenhouses and no risk assessment is required for non-target arthropods, however, for protected use other than permanent greenhouses, the risk assessment for non-target arthropods assuming the same exposure as for afield use was carried out.

In-field and off-field hazard quotient (HQ) tier 1 risk assessment

In line with ESCORT 2 guidance (2001) and Guidance Document on Terrestrial Ecotoxicology (SANCO/10329/2002) details have been provided for glass plate residue toxicity tests conducted with the indicator species *Aphidius rhopalosiphi* and *Typhlodromus pyri* and formulation Alar 85 SP. The results of these studies have been used to assess in-field and off-field Tier I risks to NTAs from the proposed uses of the representative formulation, according to the ESCORT 2 guidance.

The following equation was used to calculate the hazard quotient (HQ) for the in-field scenario:

In field-HQ = max. single application rate * MAF / LR₅₀

The in-field risk is considered acceptable if the calculated HQ is < 2.

The product is intended to be applied in an application rate of 5 x 4.25 kg daminozide/ha for field use and 5 x 7.65 kg daminozide/ha for glasshouse use, at a minimum interval of 7 days. Therefore, the multiple application factor (MAF) was set 3.0.

Table B.9.6.2-2 In- and off-field exposure of daminozide formulated product (Dazide Enhance) applied to ornamentals

Crop	Rate of use	MAF*	In-field exposure	Drift rate	Veg. distribution factor	Correction factor	Off-field exposure
Field use							
Ornamental <50 cm in height	4.25 kg a.s./ha	3	12.75 kg a.s./ha	1.75% (1 m)	10	10	0.223 kg a.s./ha
Ornamental >50 cm in height	4.25 kg a.s./ha	3	12.75 kg a.s./ha	6.59% (3 m)	10	10	0.840 kg a.s./ha
Protected use (other than permanent greenhouses)							
Ornamental <50 cm in height	7.65 kg a.s./ha	3	22.95 kg a.s./ha	1.75% (1 m)	10	10	0.402 kg a.s./ha
Ornamental >50 cm in height	7.65 kg a.s./ha	3	22.95 kg a.s./ha	6.59% (3 m)	10	10	1.512 kg a.s./ha

Table B.9.6.2-3 In-field and off-field hazard quotients (HQs) for standard laboratory terrestrial arthropods from the proposed use of daminozide

Artichokes from the proposed use of daminofenid					
Crop	Test species	LR ₅₀ ^a (kg a.s./ha)	Exposure scenario	Estimated exposure (kg a.s./ha)	HQ [Trigger = 2]
Field use					
Ornamental <50 cm in height	<i>Typhlodromus pyri</i>	> 8.50	In-field	12.75	<1.50
			Off-field	0.223	<0.026
Ornamental <50 cm in height	<i>Aphidius rhopalosiphi</i>	> 8.50	In-field	12.75	<1.50
			Off-field	0.223	<0.026
Ornamental >50 cm in height	<i>Typhlodromus pyri</i>	> 8.50	In-field	12.75	<1.50
			Off-field	0.840	<0.099
Ornamental >50 cm in height	<i>Aphidius rhopalosiphi</i>	> 8.50	In-field	12.75	<1.50
			Off-field	0.840	<0.099
Protected use (other than permanent greenhouses)					
Ornamental <50 cm in height	<i>Typhlodromus pyri</i>	> 8.50	In-field	22.95	<2.70
			Off-field	0.402	<0.047
Ornamental <50 cm in height	<i>Aphidius rhopalosiphi</i>	> 8.50	In-field	22.95	<2.70
			Off-field	0.402	<0.047
Ornamental >50 cm in height	<i>Typhlodromus pyri</i>	> 8.50	In-field	22.95	<2.70
			Off-field	1.512	<0.18
Ornamental >50 cm in height	<i>Aphidius rhopalosiphi</i>	> 8.50	In-field	22.95	<2.70
			Off-field	1.512	<0.18

All the HQ values for both *A. rhopalosiphi* and *T. pyri* for outdoor use met the trigger of 2, indicating acceptable in-field and off-field risk.

The in-field HQ values for both *A. rhopalosiphi* and *T. pyri* for protected use use did not meet the trigger of 2, indicating high risk for protected use. Further consideration is needed.

Refined in-field risk assessment for protected use (other than permanent greenhouses)

Extended laboratory studies on *T. pyri* were only available and the the refined risk assessment is presented in the table below. No additional studies were provided for *A. rhopalosiphi*.

Table B.9.6.2-4 Refined non-target arthropod in-field risk assessment for *T. pyri* for protected use (other than permanent greenhouses)

Crop	Species	Appl. rate [kg a.s./ha]	MAF	PER _{in-field} [g a.s./ha]	LR ₅₀ ; ER ₅₀ [kg a.s./ha]	Risk acceptable?
Ornamental <50 cm in height	<i>Typhlodromus pyri</i>	7.65	3.0	22.95	> 8.50	No
Ornamental >50 cm in height	<i>Typhlodromus pyri</i>	7.65	3.0	22.95	> 8.50	No

The in-field risk for both *A. rhopalosiphi* and *T. pyri* for glasshouse use was identified as high. No further refinement was provided.

Additionally, first tier laboratory studies on *Chrysoperla carnea*, *Poecilus cupreus*, *Orius laevigatus* and *Encarsia formosa*, also exposed to 8.5 kg daminozide/ha, are available. These studies demonstrated no lethal or sublethal effects of greater than 50% (ESCORT 2 trigger value) for *C. carnea*, *P. cupreus* and *O. laevigatus*. The product did result in effects of > 50% on the survival, but not the fecundity, of *E. formosa*. However, the observed toxicity was most likely caused by the sticky spray residue on the glass plates (false positive) as indicated in the Review Report (2005).

Conclusion – risk to non-target arthropods other than bees

Overall, a low risk to non-target arthropods can be concluded for the proposed field use of Dazide Enhance on ornamentals and also for permanent greenhouses. However, a high in-field risk to non-target arthropods was identified for protected uses other than permanent greenhouses.

It is noted that the risk to beneficial arthropods, used in Integrated Pest Management (IPM) in permanent greenhouses, is considered to be low, while for protected uses other than permanent greenhouses is considered high.

B.9.7 Effects on non-target soil meso- and macrofauna

B.9.7.1 Earthworms

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B.9.7.2 Effects on non-target soil meso- and macrofauna (other than earthworms)

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B.9.8 Risk assessment for non-target soil meso- and macrofauna**B.9.8.1 Earthworms****B.9.8.1.1 Summary of studies on toxicity to earthworms****Table B.8.1-1 Summary of studies on toxicity to earthworms**

Test organism	Test substance	Application method of test a.s./ OM content	Time scale	End point	Toxicity	Reference
<i>Eisenia fetida</i> [#]	Daminozide	Mixed through soil / 10% OM	Chronic	Growth, reproduction, behaviour	NOEC = 648 mg a.s./kg dws*	Pavić 2014; 87714022

* The highest concentration tested.

EPPO correction factor is not required as daminozide has log Pow value < 2.

No chronic toxicity data for methanol were provided and risk assessment was conducted. The Notifier provided the following justification:

“Supplemental acute toxicity data reviewed under the registration of methanol under REACH by the European Chemicals Agency (ECHA) demonstrates that methanol has a low toxicity to earthworms (666.67 mg/kg soil dw) and as methanol is not persistent in soil and the potential toxicity of the metabolite was also addressed within the 56-day earthworm reproduction study for daminozide; chronic exposure is not expected and no further consideration is required.”

RMS comment: Active substance daminozide degrades rapidly in soil, aerobic laboratory non-normalized DT50 values are 0.11 – 0.37 days and DT90 0.35 – 1.21 days (SFO kinetics). The polar metabolite fraction, M1, subsequently identified as methanol, degrades with aerobic laboratory non-normalized DT50 values of 4.5 – 6.2 days and DT90 of 15.0 – 20.5 days (SFO kinetics). Therefore, RMS agrees that methanol is not persistent in soil and the potential toxicity of the metabolite was also tested within the 56-day earthworm reproduction study for daminozide. No chronic risk assessment for methanol is required.

B.9.8.1.2 Risk assessment for earthworms

The earthworm risk assessment is based on the “Guidance Document on Terrestrial Ecotoxicology” (SANCO 10329/2002). Dazide Enhance intended is intended to be used as a foliar spray on ornamentals, with an application rate of 4.25 kg daminozide/ha for field use and 7.65 kg daminozide/ha for glasshouse use, in a maximum of 5 application per year, at a minimum interval of 7 days..

In Section B.8.3 worst-case PEC_{soil} values have been calculated for the proposed uses of the Dazide Enhance formulation by a fate and behaviour specialist and are summarized in the table below:

Table B.9.8.1-2 Initial max PEC_{soil} values

Compound	Ornamentals - field use (5 x 4.25 kg a.s./ha) PEC _{soil, max} [mg/kg]	Ornamentals - protected use (5 x 7.65 kg a.s./ha) PEC _{soil, max} [mg/kg]
Dazide	2.833	5.100
Methanol	0.278	0.500

* Accumulated PEC_{soil}.

Calculation of TER values

In the table below, maximum PEC_{soil} values for daminozide are compared to the chronic toxicity data to derive TERs.

Table B.9.8.1-3 TER calculations for earthworms

Test substance component	Time scale	NOEC (mg a.s./kg soil) ^a	Maximum PEC _{soil} (mg a.s./kg soil)	TER	TER Trigger
Ornamentals - field use (5 x 4.25 kg a.s./ha)					
Daminozide	Chronic	648	2.833	229	5
Ornamentals - protected use (5 x 7.65 kg a.s./ha)					
Daminozide	Chronic	648	5.100	127	5

The resulting chronic TER values are all above the relevant trigger value of 5 indicating a low risk to earthworms for all proposed uses of Dazide Enhance.

B.9.8.2 Non-target soil meso- and macrofauna (other than earthworms)

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B.9.9 Effects on soil nitrogen transformation

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B.9.9.1 Risk assessment for soil nitrogen transformation

Effects on soil nitrogen transformation are summarised in the table below.

Table B.9.9-1 Summary of data on the toxicity of daminozide to soil micro-organisms

Test	Test substance	Endpoint	Reference
Nitrogen ^{#1,2} mineralisation	Alar 85	n.a.	Mass (1987 & 1989) A.8.1.18

[#] Study evaluated in old DAR (1999).

¹ The study is not considered valid or suitable for regulatory use.

² Study summarized and evaluated in Volume 3 CP B.9 for Alar

Since no valid endpoint for soil nitrogen transformation was available no risk assessment could be performed.

B.9.10 Effects on terrestrial non-target higher plants

B.9.10.1 Summary of screening data

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B.9.10.2 Testing on non-target plants

i) A vegetative vigour test - Dazide Enhance

Reference:	Bramby-Gunary, J. (2015a) FAL 2400 - Evaluation of the phytotoxicity to non target terrestrial plant - vegetative vigour test
Report No.:	ACE-14-159
Guideline:	OECD 227 (2006)
GLP:	Yes
Previous evaluation:	Submitted for the purpose of renewal

Executive Summary:

The effects of post-emergence exposure of daminozide formulation (FAL 2400) on vegetative vigour of six species of terrestrial plant were tested in a laboratory study. Seedlings were exposed to the test item concentrations of 0.551, 1.10, 2.21, 4.41 and 8.82 g formulation/ha (equivalent to 0.469, 0.938, 1.88, 3.75 and 7.50 g a.s./ha), plus a negative control was tested in parallel.

The results demonstrated no adverse effects on the height, survival, dry weight and plant condition in any of the species tested. The ER₅₀ is greater than 7.50 g a.s./ha, the NOER is equal to the highest test dose of 7.5 g a.s./ha. Due to an average of 159% recovery of the active substance in the spray solution, the endpoints are adjusted to an ER₅₀ > 11.9 g a.s./ha and a NOER of 11.9 g a.s./ha for those species (oat, barley, carrot and radish) that were exposed to a maximum test concentration of 11.9 g a.s./ha, while cucumber and bean were accurately dosed at 7.50 g a.s./ha.

Materials and methods:**A. MATERIALS**

- 1. Test material:** Dazide Enhance (FAL 2400)
Description: White free flowing granules
Lot/Batch: 1020600013
Purity: 85.07% (w/w) (active substance: daminozide)

B. STUDY DESIGN AND METHODS**1. Test species:**

Species	Common name	Variety	Family
Monocots:			
<i>Avena sativa</i>	Oat	Firth	Poaceae
<i>Hordeum vulgare</i>	Barley	Quench	Poaceae
Dicots:			
<i>Daucus carota</i>	Carrot	Eskimo	Apiaceae
<i>Raphanus sativus</i>	Radish	Cherry belle	Brassicaceae
<i>Cucumis sativa</i>	Cucumber	Marketmore	Cucubitaceae
<i>Vicia faba</i>	Bean	Fanfare	Fabaceae

- 2. Test units:** Non-porous plastic pots (approximately 15 ± 1 cm)
Test soil: Sandy clay loam (with added slow release fertiliser)
- 3. Treatment groups:** 0.551, 1.10, 2.21, 4.41 and 8.82 g formulation/ha (equivalent to 0.469, 0.938, 1.88, 3.75 and 7.50 g a.s./ha)
- 4. Environmental conditions:**
Temperature: 14.2 - 22.7°C
Relative humidity: 51.7-83.6%
Photoperiod: 16 hours light: 8 hours darkness

5. Organism assignment and treatment:

After planting, the pots were placed in the greenhouse where the seeds were allowed to emerge and develop into seedlings. Test seedlings (BBCH 10) for each species were selected on the day of application (one seedling per pot). The selection of seedlings was based upon a visual evaluation of their similarity in both size and condition.

The experimental design consisted of a treatment group and a control group for each species. Within test groups there were five replicated experimental, with each plant contained in a separate pot. After spray mixtures were

applied, plants were maintained on greenhouse tables for the duration of the study. The replicates were arranged according to a randomised block design. Test duration was 21 days. Water lost through transpiration and evaporation was replaced by sub-irrigation with well water from the greenhouse facility.

6. Dose preparation:

The applications were made using a Mardrive cabinet track sprayer with 8004E TeeJet flat fan nozzle at 66 cm above the soil surface. The sprayer was calibrated to deliver 200 L/ha \pm 10%. Calibration was made by weight of water using 3 replicates of 5 applications to six petri dishes with an inner diameter of 86 mm. The sprayer was calibrated prior to the applications.

The highest concentration of spray solution was prepared by weighing a calculated amount of FAL 2400 (approximately 0.024 g) and diluting with tap water to obtain 500 mL volume of dose rate solution. Lower dose rates were prepared by serial dilution measuring a predetermined volume from the highest dose rate and diluting with a predetermined amount of water (150 mL) resulting in a total amount of dose rate solution of the next lower rate from which a measured volume was diluted with water resulting in a total amount of the next lower dose rate. This method of serial dilution was used for the preparation of each of the following lower rates.

Details of application dates and rates are given in the table below.

Table B.9.9.1-1 Details of application dates and rates

Identification	Common Name	Date of Application	Application vol. Rate (l/ha)	Growth stage (BBCH)	Mean Height (cm)
ACE-14-159A	Field beans	02 Dec 2014	184.70	13	30
	Cucumber	02 Dec 2014	184.70	12	18.8
ACE-14-159B	Oat	15 Dec 2014	182.98	13-14	36.9
	Barley	15 Dec 2014	182.98	13	35.7
	Carrot	15 Dec 2014	182.98	12-13	13.1
	Radish	15 Dec 2014	182.98	12-13	13.1

7. Measurements and observations:

Observations of plant condition and height were made on Days 0 (prior to application), 7, 14, and 21 (days after application). After the final observations were made, plant shoots were collected, dried, and weighed.

The height (in cm) of all live plants above soil level to the top of the tallest leaf was recorded in cm at the final assessment. All plants in one treatment pot were cut at soil level and weighed (in grams) placed in a paper bag for drying. This procedure was repeated for all the treatment pots in the five replicates of a species. Dead plants were not harvested. The height and dry weight of dead plants were not measured or weighed but were recorded as "0".

The species were dried in an oven for a minimum of 2 days. Once the plant material was dried the contents of each bag were weighed (in grams).

Spray mixtures were sampled following preparation for confirmation of their test substance concentrations. Duplicate samples were collected from the highest test rate. Initially one sample was analysed but then the second sample was analysed to verify the initial high recovery rates. The determination of daminozide in the samples was performed by high performance liquid chromatography technique (HPLC).

8. Statistics:

The descriptive statistics for calculating Analysis of Variance (AOV) Means using Agriculture Research Manager (ARM) 9 software were Least Significant Difference (LSD) with 5% significance level. As there were no statistically significant differences in final height and foliar dry weight detected below the highest tested treatment rate, it was not possible to calculate 50% Effect Rate (ER₅₀) values for any of the evaluated species. ER₅₀ values were estimated from audited mean values for dry weight and height. It was clear that ER₅₀ values for all species were above the highest rate tested so no further data analysis was required.

The No Observed Effect Rate (NOER) was the highest concentration of the test item at which no adverse effect was observed. In this test, the concentration corresponding to the NOER, had no statistically significant adverse effect ($p \leq 0.05$, LSD) when compared with the control.

Results:

A. BIOLOGICAL EFFECTS:

There were no adverse treatment-related effects on the height, survival, and dry weight of the tested non-target plants.

Results of the test are summarised by species in the table below.

Table B.9.9.1-2 Effects of daminozide formulation on mean height, dry weight and survival on day 21 of the vegetative vigour test

Species	Nominal treatment group (g a.s./ha)	Height (cm) (% of untreated control)	Dry weight (mg) (% of untreated control)	Survival (%)
Oat <i>Avena sativa</i>	Control	56.7 (n/a)	2.81 (n/a)	100
	0.469	56.1 (99)	2.81 (100)	100
	0.938	55.2 (97)	3.01 (107)	100
	1.88	55.7 (98)	2.85 (101)	100
	3.75	55.5 (98)	2.68 (95)	100
	7.50	54.6 (96)	2.70 (96)	100
Barley	Control	60.8 (n/a)	2.08 (n/a)	100

Species	Nominal treatment group (g a.s./ha)	Height (cm) (% of untreated control)	Dry weight (mg) (% of untreated control)	Survival (%)
<i>Hordeum vulgare</i>	0.469	59.9 (98)	1.95 (93)	100
	0.938	61.9 (101)	2.06 (99)	100
	1.88	58.4 (96)	1.94 (93)	100
	3.75	57.3 (94)	1.82 (88)	100
	7.50	59.2 (97)	1.76 (84)	100
Carrot <i>Daucus carot</i>	Control	26.0 (n/a)	0.880 (n/a)	100
	0.469	24.9 (96)	0.781 (89)	100
	0.938	26.8 (103)	0.846 (96)	100
	1.88	23.9 (92)	0.717 (82)	100
	3.75	25.4 (98)	0.861 (98)	100
	7.50	24.8 (96)	0.733 (83)	100
Radish <i>Raphanus sativus</i>	Control	25.1 (n/a)	2.21 (n/a)	100
	0.469	25.3 (101)	2.05 (93)	100
	0.938	25.0 (99)	2.07 (94)	100
	1.88	24.3 (97)	2.00 (90)	100
	3.75	23.4 (93)	2.00 (90)	100
	7.50	25.5 (102)	2.25 (102)	100
Cucumber <i>Cucumis sativ</i>	Control	34.0 (n/a)	3.16 (n/a)	100
	0.469	34.0 (100)	3.02 (96)	100
	0.938	32.2 (95)	2.79 (88)	100
	1.88	29.2 (86)	2.43 (77)	100
	3.75	30.1 (89)	2.80 (89)	100
	7.50	28.8 (85)	2.54 (80)	100
Bean <i>Vicia faba</i>	Control	66.0 (n/a)	3.60 (n/a)	100
	0.469	65.3 (99)	3.57 (99)	100
	0.938	64.2 (94)	3.38 (94)	100
	1.88	63.1 (93)	3.33 (93)	100
	3.75	65.8 (102)	3.69 (102)	100
	7.50	63.4 (98)	3.54 (98)	100

Based on the visual phototoxicity symptoms observed at the final assessment, the following remarks are noted:

Table B.9.9.1-3 Phytotoxicity observation

Common name	Visual Phytotoxicity Symptoms
Oat	None
Barley	Very slight stunting
Carrot	Very slight stunting
Radish	None
Cucumber	Slight stunting
Bean	None

B. TOXICITY ENDPOINTS:

The effects of test substance application on the height and dry weight of each of the test species 21 days after application resulted in ER₅₀ values > 7.50 g a.s./ha and NOERs of 7.50 g a.s./ha.

Oats, barley, carrots, and radish were exposed too much higher test concentrated based on the analysis of the spray solution samples. For these test species ER₅₀s > 11.9 g a.s./ha and NOERs of 11.9 g a.s./ha were determined based on measured spray concentration in the highest test dose samples. The endpoints are summarised in the table below.

Table B.9.9.1-4 Summary of toxicity endpoints

Species	ER ₅₀ (g a.s./ha)	NOER (g a.s./ha)
Oat	>11.9	11.9
Barley	>11.9	11.9
Carrot	>11.9	11.9
Radish	>11.9	11.9
Cucumber	> 7.50	7.50
Bean	> 7.50	7.50

C. ANALYSIS:

The recovery rate for the active substance indicate that the spray solution for ACE-14-159A was prepared to an acceptable level of accuracy (100% recovery = full intended rate). No adjustment has been made to this result.

The recovery rate for the active ingredient for the spray solution ACE-14-159B was higher than expected at 160% for the main sample and 158% for the retained reserve sample. This atypical analytical recovery result was not considered to have affected the integrity of the study as the ER₅₀ values for dry weight and height for daminozide are greater than the highest rate tested for all species thus demonstrating a worst case scenario. The dose rate has been adjusted accordingly using the mean value from the two recoveries (159%).

Recovery rates for the spray solution samples are shown below.

Table B.9.9.1-5 Analytical determination of daminozide in spray solution

Sample Number	Test item	Nominal content (mg/L)	Measured content (mg/L)	Recovery (%)
ACE-14-159A	Daminozide	42.82	40.70	105
ACE-14-159B	Daminozide	65.42	41.00	160
ACE-14-159B (Retained sample)	Daminozide	64.89	41.00	158

Validity Criteria

The validity criteria for the test were met:

- The control plants did not exhibit any phytotoxic effects.
- There was more than 90% survival in the control plants (actual value was 100%).
- The environmental conditions were identical for all the tested species.

Conclusion:

A foliar application of daminozide formulation resulted in no adverse effects on the height, survival, dry weight and plant condition in all tested plants. The ER₅₀ for all parameters were greater than 7.5 g a.s./ha for cucumber and beans, and >11.9 g a.s./ha for oat, barley carrot and radish. The NOERs were 7.5 and 11.9 g a.s./ha, respectively.

RMS comments and conclusion:

The reported study is GLP compliant and was conducted according to OECD 227 guideline (2006), except for using more plants per pot than is recommended in the guideline. It was used 4 plants per pot (15±1 cm diameter) which is appropriate for oat, barley, carrot and radish only but not for the other plant species tested (it should be used 1-2 plants of cucumber per 15 cm pot and 3 plants of bean plants per 15 cm pot). However, there was no mortality or phytotoxicity observed in control, therefore it is considered that the growth conditions were sufficient for tested plants and they did not suffer with crowding. The test results are in compliance with the guideline's validity criteria. The study is acceptable for regulatory use.

The ER₅₀ for all parameters were greater than 7.5 g a.s./ha for cucumber and beans, and >11.9 g a.s./ha for oat, barley carrot and radish. The NOERs were 7.5 and 11.9 g a.s./ha, respectively.

ii) A vegetative vigour test - Dazide Enhance

Reference:	Bramby-Gunary, J. (2015b) FAL 2400 - Evaluation of the phytotoxicity to non target terrestrial plant - vegetative vigour test
Report No.:	ACE-15-075

Guideline: OECD 227 (2006)

GLP: Yes

Previous evaluation: Submitted for the purpose of renewal

Executive Summary:

The effects of post-emergence exposure of daminozide formulation (FAL 2400) on vegetative vigour of six species of terrestrial plant were tested in a laboratory study. Seedlings were exposed to the test item concentrations of 0.0206, 0.0617, 0.185, 0.556, 1.67 and 5.00 kg formulation/ha (equivalent to 0.0175, 0.0525, 0.157, 0.472, 1.42 and 4.25 kg a.s./ha), plus a negative control was tested in parallel.

The ER₅₀ values were determined to be greater than the highest rate tested (i.e. > 4.25 kg a.s./ha) for all species tested. The most sensitive species tested was tomato with an ER₂₅ value for foliar dry weight of 0.526 kg a.s./ha and NOER of 0.157 kg a.s./ha for dry weight and height.

Materials and methods:

A. MATERIALS

1. **Test material:** Dazide Enhance (FAL 2400)
Description: White free flowing granules
Lot/Batch: 1020600013
Purity: 85.07% (w/w) (active substance: daminozide)

B. STUDY DESIGN AND METHODS

1. Test species:

Species	Common name	Variety	Family
Monocots:			
<i>Allium cepa</i>	Onion	White Lisbon	Liliaceae
<i>Triticum aestivum</i>	Wheat	Oakley	Poaceae
Dicots:			
<i>Beta vulgaris</i>	Sugar beet	Boogie	Chenopodiaceae
<i>Brassica napus</i>	Oilseed rape	Castille	Brassicaceae
<i>Glycine max</i>	Soybean	Elena	Fabaceae
<i>Lycopersicon esculentum</i>	Tomato	Mandurio	Solanaceae

2. **Test units:** Non-porous plastic pots (approximately 15 ± 1 cm)

Test soil: Sandy clay loam (with added slow release fertiliser)

3. **Treatment groups:** 0.0206, 0.0617, 0.185, 0.556, 1.67 and 5.00 kg formulation/ha (equivalent to 0.0175, 0.0525, 0.157, 0.472, 1.42 and 4.25 kg a.s./ha)

4. Environmental conditions:

Temperature: 12.6 – 29.9°C

Relative humidity: 31.3-100%

Photoperiod: 16 hours light: 8 hours darkness

5. Organism assignment and treatment:

After planting, the pots were placed in the greenhouse where the seeds were allowed to emerge and develop into seedlings. Test seedlings (BBCH 10) for each species were selected on the day of application (one seedling per pot). The selection of seedlings was based upon a visual evaluation of their similarity in both size and condition.

The experimental design consisted of a treatment group and a control group for each species. Within test groups there were five replicated experimental, with each plant contained in a separate pot. After spray mixtures were applied, plants were maintained on greenhouse tables for the duration of the study. The replicates were arranged according to a randomised block design. Test duration was 21 days. Water lost through transpiration and evaporation was replaced by sub-irrigation with well water from the greenhouse facility.

6. Dose preparation:

The applications were made using a Mardrive cabinet track sprayer with 8004E TeeJet flat fan nozzle at 66 cm above the soil surface. The sprayer was calibrated to deliver 200 L/ha \pm 10%. Calibration was made by weight of water using 3 replicates of 5 applications to six petri dishes with an inner diameter of 86 mm. The sprayer was calibrated prior to the applications.

The highest concentration of spray solution was prepared by weighing a calculated amount of FAL 2400 (14.6363 g) and diluting with tap water to obtain 600 mL volume of dose rate solution. Lower dose rates were prepared by serial dilution measuring a predetermined volume from the highest dose rate and diluting with a predetermined amount of water (150 mL) resulting in a total amount of dose rate solution of the next lower rate from which a measured volume was diluted with water resulting in a total amount of the next lower dose rate. This method of serial dilution was used for the preparation of each of the following lower rates.

Details of application dates and rates are given in the table below.

Table B.9.9.1-6 Details of application dates and rates

Identification	Common name	Date of application	Application vol. rate (L/ha)	Growth stage (BBCH)	Mean height (cm)
ACE-15-075	Onion	24 July 2015	204.97	12	18.3
	Wheat			13	22.2
	Sugar beet			12	8.8
	Oilseed rape			12	11.3
	Soybean			12	35.1
	Tomato			12	6.7

7. Measurements and observations:

Observations of plant condition and height were made on Days 0 (prior to application), 7, 14, and 21 (days after application). After the final observations were made, plant shoots were collected, dried, and weighed.

The height (in cm) of all live plants above soil level to the top of the tallest leaf was recorded in cm at the final assessment. All plants in one treatment pot were cut at soil level and weighed (in grams) placed in a paper bag for drying. This procedure was repeated for all the treatment pots in the five replicates of a species. Dead plants were not harvested. The height and dry weight of dead plants were not measured or weighed but were recorded as “0”. The species were dried in an oven for a minimum of 2 days. Once the plant material was dried the contents of each bag were weighed (in grams).

Spray mixtures were sampled following preparation for confirmation of their test substance concentrations. Duplicate samples were collected from the highest test rate but only one sample was analysed to determine the concentration of daminozide by high performance liquid chromatography technique (HPLC).

8. Statistics:

The descriptive statistics for calculating Analysis of Variance (AOV) Means using Agriculture Research Manager (ARM) 9 software were Least Significant Difference (LSD) with 5% significance level. Significant differences in mean final height and mean dry weight between treatments were indicated by an asterisk after the mean values ($p \leq 0.05$, LSD). The 25% and 50% Effect Rate (ER₂₅ and ER₅₀) values were calculated using mean values of final height and dry weight per treatment and ARM 9 software using simple probit – maximum likelihood estimation method with 95% confidence level. The mean values for each treatment group were transformed in ARM then compared to the untreated control.

The No Observed Effect Rate (NOER) was the highest concentration of FAL 2400 at which no adverse effect was observed. In this test, the concentration corresponding to the NOER, had no statistically significant adverse effect ($p \leq 0.05$, LSD) when compared with the control.

Results:**A. BIOLOGICAL EFFECTS:**

Biological results of the test are summarised by species in the table below.

Table B.9.9.1-7 Effects of daminozide formulation on mean height, dry weight and survival on day 21 of the vegetative vigour test

Test species	Nominal treatment group (kg a.s./ha)	Mean height (cm) (% of untreated control)	Mean dry weight (g) (% of untreated control)	Survival (%)
Onion	Control	23.2 (n/a)	0.382 (n/a)	100
	0.0175	23.7 (102)	0.430 (113)	100
	0.0525	21.1 (91)	0.391 (102)	100
	0.157	23.9 (103)	0.430 (113)	100
	0.472	22.7 (98)	0.443 (116)	100
	1.42	23.4 (101)	0.395 (103)	100
	4.25	21.6 (93)	0.424 (111)	100
Wheat	Control	42.8 (n/a)	2.41 (n/a)	100
	0.0175	42.9 (100)	2.44 (101)	100
	0.0525	42.2 (99)	2.32 (96)	100
	0.157	42.6 (100)	2.28 (94)	100
	0.472	42.2 (99)	2.38 (99)	100
	1.42	43.2 (101)	2.28 (94)	100
	4.25	42.1 (98)	2.17 (90)	100
Sugar beet	Control	23.7 (n/a)	2.42 (n/a)	100
	0.0175	23.1 (97)	2.36 (97)	100
	0.0525	23.0 (97)	2.39 (99)	100
	0.157	23.5 (99)	2.38 (98)	100
	0.472	23.5 (99)	2.57 (106)	100
	1.42	23.1 (98)	2.90 (120)	100
	4.25	21.0* (88)	2.81 (116)	100
Oilseed rape	Control	21.3 (n/a)	2.68 (n/a)	100
	0.0175	23.0 (108)	2.94 (110)	100
	0.0525	21.3 (100)	2.46 (92)	100
	0.157	21.8 (102)	2.71 (101)	100
	0.472	21.0 (99)	2.66 (99)	100
	1.42	19.6 (92)	2.21 (82)	100

Test species	Nominal treatment group (kg a.s./ha)	Mean height (cm) (% of untreated control)	Mean dry weight (g) (% of untreated control)	Survival (%)
	4.25	17.3* (81)	2.32 (87)	100
Soybean	Control	92.0 (n/a)	5.54 (n/a)	100
	0.0175	93.1 (101)	5.55 (100)	100
	0.0525	95.6 (104)	5.73 (104)	100
	0.157	99.9* (108)	6.14 (111)	100
	0.472	88.1 (96)	5.80 (105)	100
	1.42	76.8* (83)	5.62 (101)	100
	4.25	61.8* (67)	4.66 (84)	100
Tomato	Control	56.3 (n/a)	3.46 (n/a)	100
	0.0175	53.2 (94)	3.15 (91)	100
	0.0525	54.6 (97)	3.34 (97)	100
	0.157	54.2 (96)	3.34 (96)	100
	0.472	52.1* (92)	2.71* (78)	100
	1.42	47.6* (84)	2.14* (62)	100
	4.25	41.6* (74)	1.88* (54)	100

*Significantly different from the control.

N/A = Not applicable.

Based on the visual phototoxicity symptoms observed at the final assessment, the following remarks are noted:

Table B.9.9.1-8 Phytotoxicity observation

Common name	Visual Phytotoxicity Symptoms
Onion	None
Wheat	None
Sugar beet	None
Oilseed rape	8% at 0.0525 kg a.s./ha, 8% at 0.472 kg a.s./ha, 12% 1.42 kg a.s./ha & 47% 4.25 kg a.s./ha
Soybean	4% at 0.472 kg a.s./ha, 17% at 1.42 kg a.s./ha & 34% 4.25 kg a.s./ha
Tomato	2% at 0.0525 kg a.s./ha, 3% at 0.157, 4% at 0.472 kg a.s./ha, 20% 1.42 kg a.s./ha & 39% 4.25 kg a.s./ha

B. TOXICITY ENDPOINTS:

The high tolerance to the test item meant that ER₂₅ and ER₅₀ values based on height and foliar dry weight for all the evaluated species are greater than the highest rate, except the ER₂₅ value based on height for soybean and

ER₂₅ values based on both height and dry weight for tomato. The ER₂₅ and ER₅₀ values for the remaining species are given as >4.25 kg daminozide/ha.

The initial ER₂₅ values based on final height calculated for soybean and tomato were inconsistent with the 33% and 26% decrease in height observed at the highest treatment rate, respectively. Therefore the values were recalculated using the four highest application rates (0.157, 0.472, 1.42 and 4.25 kg daminozide/ha) which show a consistent decline in height. The recalculated ER₂₅ results are in line with the observations of the raw data and the statistical analysis.

NOER values based on dry weight for all the evaluated species except tomato and NOER values based on height for onion and wheat are equal to the highest concentration. In these cases the values are given as 4.25 kg daminozide/ha.

Table B.9.9.1-9 Summary of toxicity endpoints

Test species	ER ₅₀ (kg a.s./ha)		ER ₂₅ (kg a.s./ha)		NOER(kg a.s./ha)	
	Height	Dry weight	Height	Dry weight	Height	Dry weight
Onion	> 4.25	> 4.25	> 4.25	> 4.25	4.25	4.25
Wheat	> 4.25	> 4.25	> 4.25	> 4.25	4.25	4.25
Sugar beet	> 4.25	> 4.25	> 4.25	> 4.25	1.42	4.25
Oilseed rape	> 4.25	> 4.25	> 4.25	> 4.25	1.42	4.25
Soybean	> 4.25	> 4.25	2.66	> 4.25	0.472	4.25
Tomato	> 4.25	> 4.25	3.89	0.526	0.157	0.157

C. ANALYSIS:

The recovery rate for the active substance indicates that the spray solution was prepared to an acceptable level of accuracy (100% recovery = full intended rate). No adjustment has been made to this result.

Recovery rates for the spray solution samples are shown below.

Table B.9.9.1-10 Analytical determination of daminozide in spray solution

Sample Number	Test item	Nominal content (mg a.s./L)	Measured content (mg a.s./L)	Recovery (%)
ACE-15-075	Daminozide	20.75	21.54	104

Validity Criteria

The validity criteria for the test were met:

- The control plants did not exhibit any phytotoxic effects.
- There was more than 90% survival in the control plants (actual value was 100%).

- The environmental conditions were identical for all the tested species.

Conclusion:

The ER₅₀ values were determined to be greater than the highest rate tested (i.e. > 4.25 kg a.s./ha) for all species tested. The most sensitive species tested was tomato with an ER₂₅ value for foliar dry weight of 0.526 kg daminozide/ha and NOER of 0.157 kg daminozide/ha for dry weight and height.

RMS comments and conclusion:

The reported study is GLP compliant and was conducted according to OECD 227 guideline (2006). The test results are in compliance with the guideline's validity criteria. The study is acceptable for regulatory use.

The ER₅₀ for all parameters were greater than 4.25 kg a.s./ha, the highest concentration tested.

B.9.10.3 Risk assessment for non-target plants

B.9.10.3.1 Summary of studies for non-target plants

For evaluation of effects of daminozide on non-target plants, two vegetative vigour studies with Dazide Enhance and one vegetative vigour study and one seedling emergence study with Alar 85 WSG were available. The results of the studies are summarized in the following table.

Table B.9.9.5-1 Effects of daminozide on non-target plants

Test Substance	Study type	Most sensitive species / parameter	ER ₅₀	Reference
Dazide Enhance (FAL 2400)	Vegetative vigour	All species were equivalent / all parameters	>7.5 g a.s./ha *	Bramby-Gunary (2015a) ACE-14-159
Dazide Enhance (FAL 2400)	Vegetative vigour	Tomato / dry weight	>4.5 kg a.s./ha*	Bramby-Gunary (2015b) ACE-15-075
Alar 85 WSG	Vegetative vigour ¹	Soybean / height	>7500 ppm product; equivalent to >6413 ppm a.s.; equivalent to 13 kg a.s./ha *	Sindermann et al (2012b) 616-107
Alar 85 WSG	Seedling ¹ emergence & growth	All species were equivalent / all parameters	7500 ppm product; equivalent to >6413 ppm a.s.; equivalent to 13 kg a.s./ha *	Sindermann et al (2012a) 616-108

* The highest concentration tested.

¹ Study summarized and evaluated in Volume 3 CP B.9 for Alar

B.9.10.3.2 Risk assessment for non-target plants

The risk assessment is based on the “Guidance Document on Terrestrial Ecotoxicology”, (SANCO/10329/2002 rev2 final, 2002)³. It is restricted to off-field situations, as non-target plants are off-crop plants located outside the treated area. Spray drift from the treated areas may lead to residues of a product in off-crop areas.

The risk assessment was performed for field use (4.25 kg a.s./ha). For protected use (7.65 kg a.s./ha), the risk assessment for non-target plants should be performed assuming the same exposure as for an field use, unless it is indicated that the uses will be restricted to permanent greenhouses (based on Technical report on the outcome of the pesticides peer review meeting on general recurring issues in ecotoxicology (EFSA, 2015)). This was not indicated in the GAP. Therefore, the risk is considered low for permanent greenhouses and no risk assessment is required for non-target plants, however, for protected use other than permanent greenhouses, the risk assessment for non-target plants assuming the same exposure as for a field use was carried out.

Table B.9.9.6-1 Toxicity Exposure Ratios for terrestrial non-target plants exposed to daminozide (worst case - ornamentals >50 cm in height)

Test type	Application rate (kg a.s./ha)	Drift value ^a (%)	PER _{drift} (kg a.s./ha)	ER ₅₀ ^b (kg a.s./ha)	TER ^c	TER Trigger
Field use						
Vegetative vigour	4.25	8.02	0.34	>13	>38.24	5
Seedling emergence & growth	4.25	8.02	0.34	>13	>38.24	5
Protected use (other than permanent greenhouses)						
Vegetative vigour	7.65	8.02	0.61	>13	>21.31	5
Seedling emergence & growth	7.65	8.02	0.61	>13	>21.31	5

^a Drift estimates are based on 90th percentile values for ornamentals >50 cm in height at a 3 m buffer based on single applications (BBA 2000).

^b ER₅₀ is used to calculate the Toxicity Exposure Ratio

^c Toxicity Exposure Ratio = ER₅₀/PER_{drift}

The calculated TER values, based on basic drift values for ornamentals >50 cm in height (worst-case) single application with a 3 meter buffer exceed the trigger of 5 in all species tested for effects on seedling emergence and vegetative vigour. This indicates that there will be negligible risk to non-target plants from the proposed uses (both field and protected) of daminozide, even considering worst-case exposure scenarios without buffer mitigations.

B.9.11 Effects on other terrestrial non-target organisms

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B.9.11.1 Risk assessment for other terrestrial non-target organisms

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B.9.12 References relied on

New studies

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
CP 10.2.1.1/01	██████████ ██████████ ██████████	2009	Acute Toxicity to Common Carp (<i>Cyprinus carpio</i>) ████████████████████ Report No.0673/0006 GLP Unpublished	Y	Y	New data for AIR3 renewal	Fine Agrochemicals Ltd.
CP 10.2.1.1/02	██████████ ██████████ ██████████	2010	FAL 2400: Acute Toxicity to Common Carp (<i>Cyprinus carpio</i>) ████████████████████ Report No.41004365 GLP Unpublished	Y	Y	New data for AIR3 renewal	Fine Agrochemicals Ltd.
CP 10.2.1.2/01	Goodband, T.J. and Mullee, D.M.	2010	FAL 2400: Acute Toxicity to <i>Daphnia magna</i> Harlan Laboratories Ltd., Debyshire, UK Report No.41004366 GLP Unpublished	Y	Y	New data for AIR3 renewal	Fine Agrochemicals Ltd.
CP 10.2.1.2/02	Hernádi, D.	2007a	Acute immobilisation test with Dazide Enhance SG (Dazide 85 WSG) on <i>Daphnia magna</i> LAB. Report No. 07/482-023DA GLP Unpublished	N	Y	New data for AIR3 renewal	Fine Agrochemicals Ltd.
CP 10.2.1.3/01	Hernádi, D.	2007b	Dazide Enhance SG (Dazide 85 WSG) growth inhibition test on algae (<i>Pseudokirchneriella subcapitata</i>) LAB. Report No. 07/482-022AL GLP Unpublished	N	Y	New data for AIR3 renewal	Fine Agrochemicals Ltd.
CP/10.6.2/01	Brambury-Gunary, J.	2015a	FAL 2400 – Evaluation of the phytotoxicity to non target terrestrial plant – vegetative vigour test Agrochemex Ltd. Report No. ACE-14-159 GLP Unpublished	N	Y	New data for AIR3 renewal	Fine Agrochemicals Ltd.

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
CP/10.6.2/02	Brambury-Gunary, J.	2015b	FAL 2400 – Evaluation of the phytotoxicity to non target terrestrial plant – vegetative vigour test Agrochemex Ltd. Report No. ACE-15-075 GLP Unpublished	N	Y	New data for AIR3 renewal	Fine Agrochemicals Ltd.

Studies relied upon for the first inclusion of daminozide in Annex I to Directive 91/414/EEC and for renewal of approval under Regulation (EC) No 1107/2009

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
CP 10.3.2.2/02	Harwood et al.	2000	A laboratory evaluation of the side effects of Dazide 85 on the predatory mite <i>Typhlodromus pyri</i> . Inveresk Research Scotland Inveresk Report Number 18133 GLP, Unpublished.	N	N	Not applicable	Fine Agrochemicals Ltd..
CP 10.3.2.2/01	Taruza, S.	2001b	An extended laboratory test to determine the effect of fresh residues of Dazide 85 on the predatory mite <i>Typhlodromus pyri</i> (Acari, Phytoseiidae) Mambox-Tox Ltd. Report No. RIV-02-01 GLP Unpublished	N	N	Not applicable	Fine Agrochemicals Ltd.