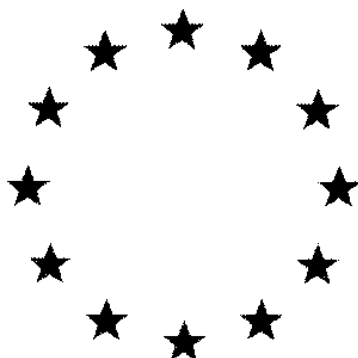


European Commission



**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) – Alar

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 in the Alar formulation (synonymous with B-NINE, Alar 85 SG, Daminozide SG). Alar is a water soluble granule formulation (SG) containing 850 g/kg daminozide (ISO); 4-(2,2-dimethylhydrazino)-4-oxobutanoic acid; N-dimethylaminosuccinamic acid ('hereafter referred to as '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 Alar (B-Nine, UBI 6899-00 SP), another formulation Dazide Enhance (FAL 2400 SP) is considered as the representative one for this renewal. Representative formulations used for Annex I inclusion were Alar 85 (UBI 2231-01 SP) and Dazide 85 (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****i) Short-term dietary toxicity in bobwhite quail – Alar 85**

Reference:	██████ (1966a) Acute dietary toxicity: Bobwhite quail Alar-85 and D-198
Report No.:	A.7.4.2.1
Guideline:	Fish and Wildlife Service (1965)
GLP:	No
Previous evaluation:	In DAR (1999)
Material and methods:	
Test material:	Alar 85 (85% daminozide)
Lot/Batch No:	C3-1296
Purity:	

An 8 day dietary toxicity test with Alar-85 (85% daminozide) was performed on *Colinus virginianus* (10 birds in 5 pens per group). Seven test concentrations, 31.6, 100, 316, 1000, 1780, 3160 and 5620 ppm, plus control. The birds were exposed to the appropriate dietary concentrations for five days, and then maintained on toxicant-free diet for an additional three-day observation period. The negative control birds received the basal diet throughout the study.

Body weights were recorded on day 0, 5 and 8 of the study. Mortality was recorded daily throughout the study. Method of statistical analysis was not stated.

Results:

Mean weight gains among bobwhite quail fed with Alar-85 were in general greater than those of birds in the negative control groups.

This was most noticeable during the initial five-day feeding period. The mean feed consumption of birds fed with the test materials Alar-85 were generally comparable to the feed consumption of birds in the negative control groups.

The 8-day LC_{50} > 5620 ppm for quail.

Table B 9.1.1-1 Mortality, average body weight and estimated food consumption of bobwhite quails

Compound	Dosage ppm	Average Weights of Birds			Survivors on Day								Food and Cpd. Consumed		Basal Food Consumed		
		Initial	5-Day	Final	0	1	2	3	4	5	6	7	8	Total	Average/ Bird/Day	Total	Average/ Bird/Day
		g.	g.	g.										g.	g.	g.	g.
Control	-	11.9	15.9	19.6	30	29	29	29	29	29	29	29	29	712.9	4.9	497.6	5.7
D, D'-DDT	316	13.1	18.0	18.3	10	10	10	10	10	10	10	10	10	214.9	4.3	121.3	4.0
	681	11.5	13.8	20.4	10	10	9	8	8	5	5	5	5	138.0	3.5	104.9	7.0
	1000	12.0	14.6	19.1	10	10	10	10	10	9	9	9	9	162.3	3.3	133.2	4.9
	1470	10.7	12.9	24.2	10	9	7	7	7	5	5	5	5	119.8	3.4	97.6	6.5
	2150	12.8	14.7	25.0	10	10	9	9	4	4	4	4	4	123.1	3.4	41.2	3.4
Alar-85	31.6	8.4	18.8	20.6	10	10	10	10	10	10	10	10	10	119.1	2.4	102.3	3.4
	100	9.4	16.5	19.8	10	10	10	10	10	10	10	10	10	142.3	2.8	129.8	4.3
	316	8.4	16.3	20.4	10	8	8	8	8	8	8	8	8	143.5	3.6	97.6	4.1
	1000	8.7	16.4	23.8	10	9	9	9	9	9	9	9	9	136.6	1.4	99.8	3.7
	1780	8.9	16.1	17.0	10	10	10	9	9	8	8	8	8	164.7	3.6	123.4	5.1
	3160	8.8	15.8	17.7	10	9	8	8	8	8	8	8	8	178.7	4.4	149.8	6.2
	5620	8.2	17.3	17.9	10	7	7	7	7	7	7	7	7	479.9*	--	--	--
D-198	31.6	8.2	15.0	17.4	10	8	8	8	8	8	8	8	8	137.2	4.3	123.1	5.1
	100	8.8	17.4	17.7	10	7	7	7	7	7	7	7	7	187.0	5.3	138.7	6.6
	316	7.7	18.2	19.0	10	10	10	10	10	10	10	10	10	167.6	3.4	144.2	4.8
	1000	9.0	17.9	18.8	10	10	10	10	10	10	10	10	10	190.9	3.8	137.8	4.6
	1780	9.2	14.6	21.5	10	10	10	10	9	9	9	9	9	199.5	4.2	129.4	4.8
	3160	8.6	17.4	22.3	10	6	6	6	6	6	6	6	6	97.8	3.3	97.6	5.4
	5620	7.5	15.4	24.1	10	10	10	10	10	8	7	7	6	158.4	3.4	94.3	4.7

*Showed excessive spillage of seed into pan due to scratching.

RMS comments and conclusion:

The reported study is non-GLP and no test guideline is stated. Several deviations from the current OCSPP 850.2200 (2012) and OECD 205 (1984) guidelines were noted. No diet analysis was performed during the study, therefore the homogeneity and stability of test substance could not be verified. Except for the room temperature, no data on housing conditions were reported (type, size and material of pen, relative humidity, photoperiod and lighting intensity). According to OECD 205 guideline, food consumption should be measured on days 0-5 and 5-8 but it is not clear from the study report when this was done. Further, method of statistical analysis was not stated.

Given a lot of shortcomings, the study is not considered valid. Moreover, it is not clear from the study report when food consumption was measured, therefore, the concentrations could not been converted to daily doses.

B.9.1.1.1 Summary of studies on toxicity to birds

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

Table B 9.1.1-2 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 bw ³	██████████ (2006) 429-104
Mallard duck (<i>Anas platyrhynchos</i>) [#]	Daminozide	Acute, oral 14 d	LD ₅₀	>2250 mg/kg bw* >4248 mg/kg bw ³	██████████, (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.	A.7.4.2.5
Bobwhite quail (<i>Colinus virginianus</i>) ^{# 1}	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

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.	██████ 2006b

				Teratogenicity: 1000 mg/kg bw/d	
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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 [REDACTED] (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 [REDACTED] (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 ([REDACTED], 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 [REDACTED] 1999, see DAR Addendum, June 2002).

NB mortality, hypoxia, convulsion/tremors seen in [REDACTED] 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>	<i>It is noted that a 90 day dog study was not available. 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. Given the likelihood of saturation of absorption increasing the dose it is unlikely to result in any greater toxicological effect. 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>

<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 (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>

Study	NOEL/NOAEL (mg/kg bw/day)	LOEL/LOAEL (mg/kg bw/day)	Effects at LOAEL	Reference	Notifier's comment:
<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</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>consumption. In addition, two animals in the 1000 mg/kg/day group aborted and were sacrificed. These 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, ptosis, 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</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>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> <p>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.</p> <p>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).</p> <p>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</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>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 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.</i></p> <p><i>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.</i></p> <p><i>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).</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><i>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 (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:

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

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:

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

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 Alar on ornamentals

Table B.7.2.1-1 Avian screening assessment for the proposed use of Alar 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 Alar 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.*

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:

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

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.

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 percentile PT</i>	<i>95th percentile 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)² 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 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)

<i>Representative species</i>	<i>FIR / bw^a</i>	<i>Refined RUD^b</i>	<i>PD</i>	<i>PT</i>	<i>MAF</i>	<i>f_{TWA}</i>	<i>DF</i>	<i>DDD (mg/kg bw)</i>	<i>End-point (mg/kg bw/d)</i>	<i>TER_{LT}</i>	<i>Trigger value</i>
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 DT_{50} in soil for the metabolite; for methanol the soil DT_{50} is 3.9 days (geomean; calculated). Note that the soil DT_{50} 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 (1.69×10^4 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	Endpoint	Ratio	Trigger value
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				rate (g a.s./ha)			
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 _c	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 _c	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 Alar

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
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.

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.

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

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 Alar 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)	TERLT	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.

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

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.

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 10.1.2-04 and 10.1.2-05, respectively, 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)

Crop/scenario	Shortcut value	MAF	DF	DDD (mg/kg bw)	Endpoint (mg/kg bw)	TER	Tigger value
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)

Representative species	FIR /bw ^a	Food type	RUD ^b	PT	PD	f _{iva}	MAF	DF ^c	DDD (mg/kg)	DDD sum	End-point (mg/kg bw)	TER	Tigger value
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

reated of mammals													
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 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 Alar 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 DT_{50} in soil for the metabolite; for methanol the soil DT_{50} is 3.9 days (geomean; calculated). Note that the soil DT_{50} 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 (1.69×10^4 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.9.2.2-6 Tier I avian drinking water assessment (puddle scenario) for the proposed use of Alar

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

^a 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).

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 $\log P_{OW} > 3$ 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 -0.77¹², 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.

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 Alar to rainbow trout (*Oncorhynchus mykiss*)

Reference:	██████████ (1966b) Acute toxicity in aqueous exposure to rainbow trout: Alar-85 and F461-75W
Report No.:	A.7.4.1.1
Guideline:	US Dep. of the Interior, Fish and Wildlife Service Guideline (1964)
GLP:	No
Previous evaluation:	In DAR (1999)
Material and methods:	
Test material:	Alar-85 (85% daminozide)
Lot/Batch No:	Not stated
Purity:	85% daminozide

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

A 96 hours toxicity test with rainbow trout (*Oncorhynchus mykiss*) was conducted using six test concentrations of Alar 85 (85% daminozide); 3.16, 10.0, 17.8, 31.6, 56.2 and 100.0 µg/L, plus control, under static conditions. Ten fish per vessel was used.

Results:

The 96 hour LC₅₀ was determined to be >0.1 mg/L. The actual test concentrations were not measured, and there was no data on the fish used or on the environmental test conditions.

Remark from previous review: Dissolved oxygen and pH were not measured. No data on fish used. Actual concentrations were not measured. The result 96h LC₅₀ >0.1 mg/L is used for risk evaluation.

RMS comments and conclusion:

The reported study is non GLP and was conducted according to US Dep. of the Interior, Fish and Wildlife Service Guideline (1964). The study report was very brief and a lot of information was missing, e.g. batch number of the tested substance, the data on fish tested and test vessels, environmental test conditions, no raw data were available. In addition, no measurements of dissolved oxygen, pH, temperature and actual concentration of the test substance were carried out. The validity criteria of the test guideline could not be checked.

The study is not considered valid.

ii) Acute toxicity of Alar to bluegill sunfish (*Lepomis macrochirus*)

Reference:	██████ (1966c) Acute toxicity in aqueous exposure to bluegill sunfish
Report No.:	A.7.4.1.2
Guideline:	US Dep. of the Interior, Fish and Wildlife Service Guideline (1964)
GLP:	No
Previous evaluation:	In DAR (1999)
Material and methods:	
Test material:	Alar-85 (85% daminozide)
Lot/Batch No:	Not stated
Purity:	85% daminozide

A 96 hours toxicity test with bluegill sunfish (*Lepomis macrochirus*) was conducted using seven test concentrations of Alar 85 (85% daminozide); 3.16, 10.0, 17.8, 31.6, 56.2, 178 and 562 µg/L, plus control, under static conditions. Ten fish per vessel was used.

Results:

The 96 hour LC₅₀ was determined to be >0.5 mg/L. The actual test concentrations were not measured, and there was no data on the fish used or on the environmental test conditions.

Remark from previous review: Dissolved oxygen and pH were not measured. No data on fish used. Actual concentrations were not measured. The result 96h LC₅₀ >0.5 mg/L is used for risk evaluation.

RMS comments and conclusion:

The reported study is non GLP and was conducted according to US Dep. of the Interior, Fish and Wildlife Service Guideline (1964). The study report was very brief and a lot of information was missing, e.g. batch number of the tested substance, the data on fish tested and test vessels, environmental test conditions, no raw data were available. In addition, no measurements of dissolved oxygen, pH, temperature and actual concentration of the test substance were carried out. The validity criteria of the test guideline could not be checked.

The study is not considered valid.

B.9.3.1.2 Acute toxicity to aquatic invertebrates

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B.9.3.1.3 Effects on aquatic algae and macrophytes

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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}	Alar 85	Acute, 96h (static)	Mortality, LC ₅₀	n.a.	██████ (1966b); A.7.4.1.1
Bluegill sunfish (<i>Lepomis macrochirus</i>) ^{#1}	Alar 85	Acute, 96h (static)	Mortality, LC ₅₀	n.a.	██████ (1966c); A.7.4.1.2
Common carp ² (<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 ² (<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	Development and growth, NOEC	1.7 (mm)	██████ (2014); 616A-123

Test organism	Test substance	Time scale, study type	Endpoint	Toxicity (mg a.s./L)	Reference
		(flow-through)			
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> ²	Dazide Enhance	Acute, 48h (static)	Immobility, EC ₅₀	60 form. 51 a.s. (nom)	Goodband & Mullee (2010); 41004366
<i>Daphnia magna</i> ²	Dazide Enhance SG	Acute, 48h (static)	Immobility, EC ₅₀	>100 form.* >85 a.s.* (nom)	Hernádi (2007); 07/482-023DA
<i>Daphnia magna</i> ^{a1}	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 ² (<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 Dazide Enhance</p> <p>^a Daminozide was tested simultaneously with formaldehyde.</p> <p>(nom) nominal concentration; (mm) mean measured concentration; form.: formulation; a.s.: active substance</p> <p>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 E_rC_{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	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

Test organism	Test substance	Time scale, study type	Endpoint	Toxicity (mg a.s./L)	Reference
<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
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 Alar 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:

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

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

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

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	P273 Avoid release to the environment 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 Alar 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:	No acute toxicity data for formulation Alar were available but aquatic acute toxicity data for all three trophic levels for similar formulation Dazide Enhance were provided. These data were used for classification of formulation Alar. The lowest endpoint was LC ₅₀ = 60 mg form./L for <i>Daphnia magna</i> : LC ₅₀ > 1 mg a.s./L, therefore, <u>no aquatic acute classification is required for formulation Alar.</u>
Aquatic chronic classification:	No chronic toxicity data for formulation Alar were provided. The chronic endpoint was available for algae for similar formulation Dazide Enhance (algal NOEC = 100 mg form./L). 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. 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 Alar.</u>

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****B.9.5.1.1 Acute toxicity to bees****i) Acute oral and contact toxicity to bees**

Reference:	Cole, J. (1985) The acute oral and contact toxicity to honey bees of Alar 85
Report No.:	A.7.4.2.7
Guideline:	WD D3 of the UK Pesticides Safety Precautions Scheme “Laboratory testing for toxicity to honey bees” (1985)
GLP:	Yes
Previous evaluation:	In DAR (1999)
Material and methods:	
Test material:	Alar 85 (daminozide 85%)
Lot/Batch No:	Not stated
Purity:	Not stated

The contact and oral toxicity of Alar 85 to honeybees, *Apis mellifera*, was determined under laboratory conditions for 48 hours. Ten bees were placed in each cage. Ten cages were used for each contact and oral test. As a control, four cages were used in each test. One concentration of 100 µg/bee was tested in both tests. No toxic standard was used. Tests were conducted in the darkness at 24°C ± 1° in cages made of 2 mm aperture wire mesh. The cages were cylinders 11.5 cm long and 4.0 cm in diameter. Relative humidity was not reported. After 24 and 48 hours, the number of dead bees was counted.

Contact toxicity: It was carried out by applying 1.0 µL droplets of aqueous solutions of Alar 85 to each bee (on the ventral surface of the thorax) using a micrometer syringe. The bees were then replaced in the cage and supplied with the 20% w/v sucrose solution. Four cages of bees were treated with water only as controls.

Oral toxicity: A 0.2 ml volume of a solution of the appropriate concentration of the test material in 20% sucrose in water was presented to each group of ten bees in a small glass tube. When the bees had taken all the test solutions (about 3 hours) the dosage tubes were replaced with tubes containing 20% w/v sucrose solution.

Results:

After oral dosing a maximum of 33% mortality was reported. The reported LC₅₀ oral and contact was > 100 µg/bee. Mortality of bees is presented in the table below.

Table B.9.5.1-1 Mortality of bees after oral and contact treatment with Alar 85

	Group	Oral		Contact	
		24 hours	48 hours	24 hours	48 hours
Treated	1	2	4	1	2
	2	0	1	0	1
	3	2	2	2	2
	4	1	5	0	3
	5	1	3	0	3
	6	3	3	3	5
	7	2	6	0	2
	8	1	2	3	4
	9	0	3	1	4
	10	1	4	1	1
		13%	33%	11%	27%
Untreated	1	0	0	0	1
	2	1	1	0	0
	3	0	2	0	0
	4	1	1	1	2
		5%	10%	2.5%	7.5%

RMS comments and conclusion:

The reported study is GLP compliant and was conducted according to UK guideline (1985). It is also in line with the current OECD 213 and 214 guidelines, except for several deviations. According to the OECD 213, the amount of treated diet consumed per group should be monitored. It is not clear from the study report if this procedure was followed. In addition, OECD 213 recommends use of 50% (w/v) sucrose solution as food while 20% (w/v) sucrose solution was used in the study. Further, the mortality was assessed 48 hours after start of the test while OECD 213 and 214 recommend that mortality should be recorded also at 4 h and at 24 h. It is noted that no toxic standard was used and relative humidity was not reported.

The test results of the study are partly in compliance with the OECD 213 and 214 guidelines' validity criteria (mortality in control less than 10%; but criterion on LD₅₀ of toxic standard could not be checked).

The 48-hour oral and contact LD₅₀ is >100 µg formulation/bee (equivalent to >85µg a.s./bee).

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 *Aphidius rhopalosiphi* in laboratory test

Reference:	Baxter, I. (1999a) A laboratory test to determine the effect of Alar 85 SP (an 850 g/kg water soluble powder formulation of daminozide) on the parasitic wasp, <i>Aphidius rhopalosiphi</i>
Report No.:	UNI-99-9
Guideline:	IOBC/WPRS Mead-Briggs (1992)
GLP:	Yes
Previous evaluation:	Not evaluated in DAR (1999) or Addenda, but included in the Review Report for Daminozide, 2005 (SANCO/3043/99 – Final)
Material and methods:	
Test material:	Alar 85 SP (850 g/kg daminozide)
Lot/Batch No:	SI 6957
Purity:	84.4%

A study to determine the effects of the water-soluble (Alar 85 SP) powder formulation of daminozide (850 g/kg) on the parasitic wasp, *Aphidius rhopalosiphi* was performed. A group of forty *A. rhopalosiphi* were exposed to Alar 85 SP (850 g daminozide/kg) in four replicate test units. Each test unit consisted of four treated glass plates connected to an aluminium frame with mesh covered ventilation holes and an aperture for introduction of wasps and food source. To prevent the build-up of daminozide residues, air was drawn from each unit using a small aquarium pump. The glass plates were treated with Alar 85 SP at a rate equivalent to 10 kg/ha (\approx 8500 g a.s./ha) in a dilution equivalent to 200 L/ha using a Potter laboratory spray tower. Four replicate units were also treated with the toxic reference dimethoate at a rate of 0.17 g a.s./ha in 200 L/ha of water. An additional four replicate units treated with tap water at a rate equivalent to 200 L/ha were established as the control.

After allowing around 1 hour for drying of the residues on the glass plates, the units were assembled and the wasps (minimum of 5 females) were introduced into each unit using an aspirator. The access hole was then bunged with cotton wool soaked in a 1:3 honey:water solution to act as a food source. The test units were placed in a controlled environment room for a period of 48 hours. Assessments of treatment-related effects were made 2, 24 and 48 hours after test initiation.

Fecundity assessments were carried out on the surviving females after 48 hours. Fifteen female wasps from the test substance and control plots were transferred to individual pots of barley seedlings previously infested with >100 host aphids (*R. padi* and *M. dirhodum*). After 24 hours the adult wasps were removed and the aphid infested plants maintained for a further 12 days and the number of aphid mummies on each plant were recorded. The percentage mortality in each treatment was calculated and corrected for control mortality using Abbot's formula (Abbot, 1925). The number of dead wasps in the test substance and control treatments were analysed by a *t-test* for unmatched pairs (Fowler and Cohen, 1990) using validated statistical software.

Results:

There was no mortality in the control group during the study. After 2, 24 and 48 hours exposure to Alar 85 SP 0%, 2.5% and 12.5% of the test wasps were moribund/dead, respectively. The Abbott-corrected mortality in the Alar 85 SP treatment was 10%, there was no statistical difference compared to the control $p > 0.05$. In the group exposed to dimethoate, there was 100% mortality by 24 hours after test initiation. The mean number of mummies produced per female wasp was 21.1 and 21.6 in the control and Alar 85 SP treatments respectively.

Table B.9.5.2-1 Biological results

Treatment	24h	48h	Abbott-corrected % mortality after 48 h	Mean number of mummies per female (n=15)
control	0	2.5	-	21.1
Alar 85 SP	2.5	12.5	10	22.6
dimethoate	100	100	100	-

Following exposure to Alar 85 SP at a treatment rate of 10 kg/ha \equiv 8500 g a.s/ha, mortality of the parasitic wasp, *Aphidius rhopalosiphi* was 10% after 48 hours. The mean number of mummies produced per female wasp was 21.1 (2% more compared to control). It can therefore be concluded that, under extreme worst-case laboratory test conditions, Alar 85 SP: is classified as 'harmless' to this species.

Remark from previous review: The results are used for risk assessment.

RMS comments and conclusion:

The reported study is GLP compliant and was conducted according to guideline by Mead-Briggs (1992). It is also in line with the current guideline Mead-Briggs *et al.* (2000). The test results are in compliance with the guidelines' validity criteria (mortality in the control treatment over 48 hours should not exceed 13%: actual value was 2.5% mortality at 48 h; corrected mortality in the toxic reference treatment should exceed 60% at 48 h: actual value was 100%; mean number of mummies in the control treatment should be > 5.0 per female: actual mean value was 21.1 mummies per surviving female in the control; should not be more than two zero values in the control treatment: there was no zero value in the control). The study is acceptable for regulatory use.

The 48-hour LR_{50} and 14-day ER_{50} is >10 kg formulation/ha (equivalent to 8.5 kg a.s./ha).

ii) Effects on *Typhlodromus pyri* in laboratory test

Reference:	Vinall, S. (1999) A laboratory test to determine the effect of Alar 85 SP (an 850 g/kg water-soluble powder formulation of daminozide) on the predatory mite, <i>Typhlodromus pyri</i>
Report No.:	UNI-99-8
Guideline:	IOBC/WPRS Overmeer (1988)
GLP:	Yes
Previous evaluation:	Not evaluated in DAR (1999) or Addenda, but included in the Review Report for Daminozide, 2005 (SANCO/3043/99 – Final)
Material and methods:	
Test material:	Alar 85 SP (850 g/kg daminozide)
Lot/Batch No:	SI 6957
Purity:	84.4%

A study to determine the effects of the water-soluble (Alar 85 SP) powder formulation of daminozide (850 g/kg) on the predatory mite, *Typhlodromus pyri* was performed. A group of one hundred protonymph *T. pyri* were exposed to Alar 85 SP (850 g a.s./kg) in five replicate test units. Each test unit consisted of a glass Petri dish, a central disc of which (2.8 cm) was treated, covered by a 9 cm diameter filter paper with a central hole (3 cm) removed. The filter paper was kept moist with additional filter wicks leading to a water reservoir. The moist paper acted as a source of drinking water and a barrier. To provide a further barrier, a circle of Tanglefoot gel was applied around the central hole. Small pieces of broken glass cover slips were used (also treated) to provide shelter. The glass dishes were treated with Alar 85 SP at a rate equivalent to 10 kg/ha (\cong 8500 g a.s./ha) in a dilution equivalent to 200 L/ha using a Potter laboratory spray tower. Five replicate units were also treated with the toxic reference dimethoate at a rate of 34 g a.s./ha in 200 L/ha of water. An additional five replicate units treated with tap water at a rate equivalent to 200 L/ha were established as the control.

After allowing ca. 60-90 minutes for drying of the residues on the glass dishes, the units were assembled and the protonymphs introduced into each unit using a fine brush. Broad bean pollen was sprinkled into the central hole to act as a food source and replenished as required during the week. The test units were placed in a controlled environment room for a period of 7 days (23-26 °C, 55-77% relative humidity and 16 hour photoperiod of 1190 lux).

Assessments of mortality were made 1 and 7 days after treatment. In addition on Day 7 the number of male and female mites was recorded, plus the number of eggs that had already been produced was noted. To assess fecundity after 7 days the surviving mites were transferred to fresh test units, fresh bean pollen was supplied as food. The test units were set up under the same environmental conditions as before. The total number of eggs (including live and juvenile) were determined up to 14 days after treatment.

Abbott formula used to correct for control mortality. No other statistical analysis considered necessary. Validity criteria based on mortality in the control and toxic reference groups met. For fecundity the *t-test* for unmatched pairs was used.

Results:

Mortality in the control group reached 2% 7 days after introduction of the protonymphs. Corrected mortality in the Alar 85 SP and dimethoate groups was 11.3% and 100% after 7 days exposure, respectively. The mean number of eggs produced per female was calculated to be 7.2 and 3.9 in the control and Alar 85 SP treatment groups respectively. There was a statistical difference when comparing the number of eggs per female in each replicate unit for the treated and control units $p < 0.001$.

Table B.9.5.2-2 Biological results

Treatment	Mean % mortality 7 DAT	Mean number eggs per female
Control	3	7.2
Alar 85 SP	14	3.9
Dimethoate	100	No survivors

Following exposure to Alar 85 SP at an application rate of 10 kg/ha (\cong 8500 g a.s./ha), mortality of the predatory mite, *Typhlodromus pyri* was 14% after 7 days. The mean number of eggs per female after 14 days was 3.9 (i.e. 46% less compared to control). According to EPPO guidelines it can therefore be concluded that, under extreme worst-case laboratory test conditions, Alar 85 SP is classified as ‘harmful’ to this species.

Remark from previous review: The results are used for risk assessment.

RMS comments and conclusion:

The reported study is GLP compliant and 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 3%; corrected mortality in the toxic reference treatment should be 50-100%: actual value was 100% 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 7.2);).

The study is acceptable for regulatory use.

The 7-day LR_{50} and the 14-day ER_{50} is >10 kg formulation/ha (equivalent to 8.5 kg a.s./ha).

iii) Effects on *Encarsia formosa* in laboratory test

Reference:	Halsall, N. (2000) A laboratory test to determine the effect of Alar 85 SP (an 850 g/kg water-soluble powder formulation of daminozide) on the whitefly parasitoid <i>Encarsia formosa</i> Gahan (Hymenoptera, Aphelinidae)
Report No.:	UNI-00-2
Guideline:	EPPO No. 142 (1989); ESCORT Barrett (1994)
GLP:	Yes
Previous evaluation:	In Addendum 1 (2002)

Executive Summary

A study to determine the effects of Alar 85 SP (850 g/kg daminozide) on the parasitic wasp, *Encarsia formosa* was performed. Test material was applied using twice the maximum application rate (equivalent to 2 x 4.25 kg a.s./ha (i.e. 10 kg product/ha)). Wasps (females only) < 24 hours after their emergence were used for the bioassays. The test system consisted of treated glass plates fitted to a square aluminium frame (10 cm x 10 cm) with suitable holes for ventilation. The glass plates were sprayed on one surface to achieve a water volume of 200 L/ha. Once dry, the plates were used to line the floor and ceiling of shallow test arenas into which 15 adult female wasps were transferred. Four replicate arenas were used per treatment. Bean leaves infested with 100-300 whitefly scales at a suitable age for parasitisation were offered to the wasps for ovodeposition at 1, 3 and 6 days. Mortality and fecundity of the wasps were assessed 6 days after treatment. Fecundity assessment was done by recording the number of parasitised whitefly scales per female. Mean mortality after 6 days was 18% in the control and 100% in the reference treatment. Mean mortality in the Alar 85 SP treatment was 85%. After Abbott's correction, mortality was 82% which was significantly different from the control. In the control and Alar 85 SP treatment, a mean of 245 and 212 parasitised scales per replicate, respectively, were produced during the bioassay. When the data were adjusted to take account of the numbers of live females, the mean number of parasitised scales per wasp alive at each assessment was 18.2 in the control and 17.8 in the Alar 85 SP treatment. The results for fecundity were not significantly different from the control. Compared to the control, fecundity was reduced by 2.2 %.

Material and methods:**A. MATERIALS**

1. **Test material:** Alar 85 SP
Formulation: Water soluble powder
Lot/Batch: SI 6957
Purity: 84.4% w/w
Density: Not stated
2. **Reference item:** BASF Dimethoate 40
Formulation: Emulsifiable concentrate
Lot/Batch: 082

Purity: 400 g/L
Density: Not stated

B. STUDY DESIGN AND METHODS

1. Test animals: *Encarsia formosa* Gahan (Hymenoptera, Aphelinidae)

Age: <24 hours old

Source: Biological Crop Protection, Wye Kent

Acclimation: Not stated

Diet: Honey + gelatin medium (210 g + 3 g, respectively, diluted in 90 mL distilled water)

2. Test units: Treated glass plates fitted to a square frame (10 x 10 cm) with access and ventilation holes. Sprayed glass plates were fitted to the top and bottom of the frame. One glass plate had a 55 mm diameter central hole and was normally covered with a third, outer glass plate but this plate was removed and bean leaves offered to the wasps for the fecundity experiment.

3. Environmental conditions:

Temperature: 21-24°C

Relative humidity: 38-100%

Photoperiod: 16 hours light: 8 hours darkness (1139 lux)

4. Animal assignment and treatment:

Treatments were applied to glass plates at an application rate of 10 kg product/ha. Glass plates were sprayed, left to dry and the treatment arenas assembled and connected to air flow. Fifteen adult female wasps were then transferred into each test arena. Thin stripes of the honey-agar food gel were drawn onto pieces of paper (5mm x 15 mm) and one of these inserted into each arena. Water and honey:water (1:3) were also provided on separate cotton wool bungs into each arena. The honey-agar was replenished at the time of each mortality/fecundity assessment i.e. 1, 3 and 6 days after treatment.

Bean leaves infested with 100-300 whitefly scales at a suitable age for parasitisation were offered to the wasps at 1, 3 and 6 days after treatment (DAT). The outer glass plate was removed and the infested leaf, still attached to the plant, was sandwiched against the 5mm diameter hole using synthetic foam padding. The leaves were left in place for 4 hours before being removed.

Spray solutions were applied to glass plates using a Potter Laboratory Spray Tower.

5. Dose preparation:

5 g product diluted to 100 mL with water.

6. Measurements and observations:

The condition of the wasps was assessed at 1, 3 and 6 DAT, just prior to the fecundity assessments being initiated. The insects were recorded as being:

Live: alive and apparently unaffected

Moribund: on their back or side but still twitching

Dead: no longer moving

For the fecundity assessments, the number of black parasitised whitefly scales was counted approximately 12-15 days after their introduction to the test arenas.

7. Statistics:

For each treatment, the results were expressed in terms of percentage mortality of wasps 6 DAT (data corrected for any control treatment losses using Abbott's formula) and the mean number of parasitised whitefly scales per female for each fecundity assessment. The results for mortality were analysed by t-test for unmatched pairs. Prior to analysis, the percentage mortality data were angularly transformed (square root/arcsine) to help normalise their distribution. The results for parasitisation were analysed on a date-by-date basis (1 and 3 DAT only) by t-test for unmatched pairs. All analyses were carried out using SPSS software.

Results:

A. BIOLOGICAL EFFECTS

The mortality in the control treatment was 18% at 6 DAT compared to 85% in the Alar 85 SP treatment and 100% mortality with the reference treatment. When adjusted for control treatment losses, the corrected mortality in the Alar 85 SP treatment was 82% and the difference between the treatments was statistically significant (t-test, $P < 0.01$).

In the control treatment, the mean total number of parasitised scales produced per replicate was 245 (i.e. individual means of 146, 33 and 66 per replicate in the 1, 3 and 6 DAT assessments, respectively). When adjusted to take into account the numbers of live female wasps at the time of each assessment (a mean of 15, 13 and 11 per replicate at 1, 3 and 6 DAT, respectively), the resultant mean total number of scales parasitised per wasp was 18.2 (i.e. means of 9.7, 2.5 and 6.0 per replicate in the respective assessments).

In the Alar 85 SP treatment, the mean total number of parasitised scales produced per replicate was 212 (i.e. individual means of 103, 41 and 68 per replicate in the 1, 3 and 6 DAT assessments, respectively). When adjusted to take into account the numbers of live female wasps at the time of each assessment (a mean of 15, 11 and 9 per replicate at 1, 3 and 6 DAT, respectively), the resultant mean total number of scales parasitised per wasp was 17.8 (i.e. means of 6.9, 3.3 and 7.6 per replicate in the respective assessments).

These results suggest that the exposure of wasps to Alar 85 SP did not affect the fecundity of the insects. The results for the test material and the control treatments did not differ significantly in the 1 and 3 DAT assays (t-test, $P > 0.05$). The data for the 6 DAT assay was not considered appropriate for analysis due to the single replicate evaluated in the test material treatment.

A summary of the results is presented in Table 8.3.2.1/02-01 below.

Table B.9.5.2-3 Summary of effects on mortality and fecundity

Treatment	Mortality after 6 days (%)		Fecundity	
	Mortality	Corrected mortality	Mean number of parasitized scales per replicate	Mean number of scales parasitized per wasp alive at each assessment
Control	18	-	245	18.2
Alar 85 SP, 10 kg product/ha	85	82	212	17.8
Dimethoate 40	100	0.0	-	-

B. DEFICIENCIES

The following protocol deviations were noted:

- Use of deionised water in the control treatment instead of tap water
- Preparation of whitefly-infested plants in a culture box for three days instead of two days. Culture boxes kept in a controlled environment room at 14-25°C, 20-25°C and 18-23°C instead of the stated 19-25°C.
- Adult wasps collected in glass tubes instead of plastic pots.
- Provision of water and a honey:water mix as well as a honey:agar food gel.
- Residues of the test material left to dry for 1 hour 45 minutes instead of the stated 1 hour.
- Relative humidity in the test room of 38-100% instead of the stated 46-85%

None of these deviations are considered to affect the integrity of the study.

Conclusion:

Under the test conditions, residues of Alar 85 SP, applied at 10 kg product/ha, were harmful to the wasp *Encarsia formosa*, resulting in 82% corrected mortality. However, the Alar 85 SP treatment did not significantly affect the fecundity of the exposed test insects. The mortality is most likely to be caused by the sticky spray residues seen on the glass plates (false positive).

Remark from previous review: The results are used for risk assessment.

Remark in the Review Report for Daminozide (SANCO/3043/99 – Final (15 February 2005): High mortality in the treatment group most likely caused by the sticky spray residue on the glass plates (false positive). It contradicts the harmless classification observed in the Tier 1 test with *Aphidius rophalosiphi*.

RMS comments and conclusion:

The reported study is GLP compliant and was conducted according to EPPO No. 142 guideline (1989). The deviation from the guideline in relative humidity has been noted. The range recorded in the test was 38-100%, while the guidelines recommends 70-80%. However, it is not considered to have an impact on validity of the study. The test results are in compliance with the guideline's validity criteria (mortality in the control treatment over 6 days should not exceed 20%: actual value was 18% mortality; corrected mortality in the toxic reference treatment should exceed 60% over 6 days: actual value was 100%). The study is acceptable for regulatory use.

iv) Effects on *Orius laevigatus* in laboratory test

Reference:	Vinall, S. (2000) A laboratory test to determine the effect of Alar 85 SP (an 850 g/kg water-soluble powder formulation of daminozide) on the predatory bug <i>Orius laevigatus</i>
Report No.:	UNI-00-3
Guideline:	Van De Viere et al. (1996); IOBC/WPRS Bakker (1992)
GLP:	Yes
Previous evaluation:	In Addendum 1 (2002)

Executive Summary

A study to determine the effects of Alar 85 SP (850 g/kg daminozide) on the predatory bug, *Orius laevigatus* was performed. Test conditions using twice the maximum application rate equivalent to 2x 4.25 kg a.s./ha (i.e. 10 kg product/ha) were used on three to four day old nymphs. The bugs were exposed to fresh treatment residues in small closed arenas based on the 'coffin cell' described by Bakker *et al.* (1992). For the initial assessments of mortality, five replicates arenas per treatment with 20 nymphs each were used with effect assessments made 1, 3, 7 and 10 days after treatment. For assessments of the reproductive capacity, surviving bugs were transferred to the fecundity arenas, provided with untreated moth eggs and left for 4 days to allow to mature and to acclimatise to the conditions. Three replicate pots containing 10 adult (5 males and 5 females) were then prepared for each treatment and the number of eggs laid in each leaf was assessed when the leaves were replaced after each 3-day interval during a period of 9 days. Mean mortality was 17% in the control and 100% in the reference treatment after 10 days. Mean mortality in the Alar 85 SP treatment was 1%. For the fecundity assessments, the mean number of eggs per female per day over a period of nine days was 7.5 and 7.9 in the control and in the Alar 85 SP treatment, respectively. Results did not differ significantly between treatments. The hatching success of eggs was the same in both treatments (i.e. 73%) and the number of viable eggs per female per day was 5.5 and 5.7 in the control and the Alar 85 SP treatment, respectively. Compared to the control treatment, fecundity was increased in the Alar 85 SP treatment by 3.6%.

Material and methods:**A. MATERIALS**

1. **Test material:** Alar 85 SP
Formulation: Water soluble powder
Lot/Batch: SI 6957
Purity: 84.4% w/w
Density: Not stated
2. **Reference item:** BASF Dimethoate 40
Formulation: Emulsifiable concentrate
Lot/Batch: 082
Purity: 400 g/L
Density: Not stated

B. STUDY DESIGN AND METHODS

1. **Test animals:** *Orius laevigatus*
Age: 3-4 days old
Source: Biological Crop Protection, Wye Kent
Acclimation: Not stated
Diet: Moth eggs
2. **Test units:** Small closed arenas based on the ‘coffin cell’ (polytetrafluoroethylene [PTFE] rectangular frame sandwiched between two glass plates) described by Bakker *et al.* (1992) were used for the mortality assessments. For the fecundity assessments, test insects were held in 9 x cm plastic pots) with clip-on lids with a centre replaced with nylon netting to allow ventilation.
3. **Environmental conditions:**
Temperature: 21-24°C (mortality); 20-24°C (fecundity)
Relative humidity: 51-74% (mortality); 47-85% (fecundity)
Photoperiod: 16 hours light: 8 hours darkness (2900 lux)
4. **Animal assignment and treatment:**
For the mortality assessments, glass plates were sprayed on one surface and left to dry. The PTFE sheets and ventilation bungs were also sprayed. Once dry, the units were assembled and twenty nymphs of *O. laevigatus* (i.e. five replicates, 100 per treatment) were transferred into each test arena. Moth eggs were added to the arenas as food and then replenished at 1-3 day intervals.

After 10 days, the surviving bugs were added to the fecundity arenas and left for four days to acclimatise to their conditions. Three replicate pots were prepared for each treatment with an equal sex ratio. A fresh, untreated sweet pepper leaf was placed in each pot at the start of the assessment and this was replaced twice more at 3 day intervals. Oviposition assessments were made over a 9 day period.

Spray solutions were applied to glass plates, PTFE frame and tops of ventilation bungs using a Potter Laboratory Spray Tower.

5. Dose preparation:

5.003 g product diluted to 100 mL with water.

6. Measurements and observations:

The condition of the bugs was assessed at 1, 3, 7 and 10 days after treatment (DAT). The bugs were recorded as being:

Live: alive and apparently unaffected

Moribund: on their back or side but still twitching

Dead: no longer moving

Unseen: bugs not seen

For the fecundity assessments, the number of eggs laid in each leaf was assessed when the leaves were replaced at 3 day intervals. The eggs were expected to hatch from approximately one day after leaf change, therefore the leaf was checked at daily intervals until no further eggs were seen to hatch.

7. Statistics:

For each treatment, the results were expressed in terms of percentage mortality of bugs 10 DAT (data corrected for any control treatment losses using Abbott's formula) and the mean number of eggs produced per female per day and the mean percentage of eggs that hatched. No statistical analysis of the mortality data was considered necessary. The results of the fecundity assessments were analysed by t-test for unmatched pairs (Fowler, and Chohen, 1990).

Results:

A. BIOLOGICAL EFFECTS

The mortality in the control treatment was 17% at 10 DAT compared to 14% in the Alar 85 SP treatment and 100% mortality with the reference treatment. When adjusted for control treatment losses, the corrected mortality in the Alar 85 SP treatment was 0%.

In the control treatment, there was a mean of 7.5 eggs per female per day over the nine day assessment period. In the Alar 85 SP treatment, there was a mean of 7.9 eggs per female per day. For each of the batches of eggs collected over 3 day intervals, the number of eggs produced per female did not differ significantly between treatments (t-test, $P > 0.05$). The hatching success of eggs was the same in the two treatments, being 73%. The mean number of viable eggs per female per day was estimated to be 5.5 in the control and 5.7 in the Alar 85 SP treatment.

A summary of the results is presented in Table B9.5.2-4 below.

Table B.9.5.2-4 Summary of effects on mortality and fecundity

Treatment	Mortality after 10 days (%)		Fecundity		
	Mortality	Corrected mortality	Mean number eggs/female/day	Mean % hatch	Mean number viable eggs/female/day
Control	17	-	7.5	73	5.5
Alar 85 SP, 10 kg product/ha	14	0	7.9	73	5.7
Dimethoate 40	100	100	N/A	N/A	N/A

B. DEFICIENCIES

The following protocol deviations occurred:

- Culture boxes were kept in a controlled environment room at 18.5°C instead of the stated 19-25°C.

This deviation is not considered to affect the integrity of the study.

Conclusion:

Under the test conditions, Alar 85 SP was harmless to the bug, *Orius laevigatus*, when applied at 10 kg product/ha.

Remark from previous review: Results are used for risk assessment.

RMS comments and conclusion:

The reported study is GLP compliant and was conducted according to the Van De Viere et al. (1996) and IOBC/WPRS Bakker (1992) guidelines. It is also in line with the current guideline Bakker *et al.* (2000).

The study results meet the guidelines' validity criteria (mortality in the control treatment should not exceed 25%: actual value was 17%; corrected mortality in the toxic reference treatment should be 40-100%: actual value was 100%; mean number of eggs in the control treatment should be > 2.0 per female per day: actual mean value was

7.5 mummies per surviving female in the control; should not be more than five zero values in the control treatment: there was no zero value in the control; a minimum of 70% of eggs in the control should hatch successfully: actual value was 73).

The study is acceptable for regulatory use.

The 10-day LR₅₀ and ER₅₀ is >10 kg formulation/ha (equivalent to 8.5 kg a.s./ha).

v) Effects on *Poecilus cupreus* in laboratory test

Reference:	Baxter, I. (1999b) A laboratory test to determine the effect of Alar 85 SP (an 850 g/kg water soluble formulation of daminozide) on the ground beetle, <i>Poecilus cupreus</i>
Report No.:	UNI-99-10
Guideline:	IOBC/WPRS Heimbach (1991)
GLP:	Yes
Previous evaluation:	Not evaluated in DAR (1999) or Addenda, but included in the Review Report for Daminozide, 2005 (SANCO/3043/99 – Final)
Material and methods:	
Test material:	Alar 85 SP (850 g/kg daminozide)
Lot/Batch No:	SI 6957
Purity:	84.4%

A study to determine the effects of the water-soluble (Alar 85 SP) powder formulation of daminozide (850 g/kg) on the ground beetle, *Poecilus cupreus* was performed. A group of 30 adult *P. cupreus* were exposed to Alar 85 SP (850 g a.s/kg) in five replicate test units. Each test unit consisted of a disposable plastic box (13 x 18 x 6 cm) lined with clean silica sand wetted to 70% of its pre-determined water holding capacity with distilled water. In order to provide ventilation a 12 x 7.5 cm hole was cut into the lid of each box and covered with fine mesh netting. Three days prior to treatment, three females and three males were introduced to each test unit and acclimatised to the test conditions in the absence of food. After the acclimation period, the sand was re-wetted to 70% holding capacity and the test units treated by applying Alar 85 SP at a rate equivalent to 10 kg/ha (\cong 8500 g a.s/ha) in a dilution equivalent to 400 L/ha using an Azo compressed air sprayer. Just prior to treatment six freeze-killed house fly pupae (pierced at one end) were added to each unit as a food source. Application was made outdoors, during which the beetles were seen to be on the sand surface. After spraying the internal walls of the boxes were wiped to remove wet spray deposits and the lids replaced. Five replicate units were also treated with the toxic reference dimethoate at a rate of 300 g a.s./ha in 400 L/ha of water. An additional five replicate units treated with tap water at a rate equivalent to 400 L/ha were established as the control. The boxes were then placed in a controlled environment room maintained at 19-21 °C, 53-78% relative humidity and a photoperiod of 16 hours and 1400 lux, for a period of 14 days.

Assessments of mortality were made 2 and 4 hours after treatment and then at 1, 2, 4, 7, 10 and 14 days post-treatment. One fly pupa was provided for each surviving beetle (i.e. those classified as alive, affected or moribund) on days 0, 2, 4, 7 and 10 and the number of pupae remaining uneaten was recorded at 2, 4, 7, 10 and 14 days after treatment, at which time fresh food was provided and partially eaten pupae removed.

Statistical analysis of survival data (100% survival in Alar 85 SP and control groups) and food consumption was not considered necessary).

Results:

The results of the test are summarized in the table below.

Table B.9.5.2-5 Biological results

Treatment	% mortality of beetles after 14 days	Mean number of fly pupae consumed per beetle
control	0	4.83
Alar 85 SP	0	4.90
Dimethoate 40	100	-

There was no mortality (0%) in the control group or in the group exposed to Alar 85 SP. Mortality in the toxic reference group was 100% within 2 days of treatment. Feeding behaviour of *P. cupreus* exposed to Alar 85 SP was not adversely affected. Mean number of fly pupae consumed was 4.83 and 4.90/beetle in the control and Alar 85 SP groups, respectively. This represented 97-98% of pupae offered in the control and Alar 85 SP exposed beetles.

Following exposure to Alar 85 SP at a treatment rate equivalent to 10 kg/ha (\cong 8500 g a.s./ha for 14 days, there was no adverse effect on survival or feeding of the ground beetle, *Poecilius cupreus*. It can therefore be concluded that, under extreme worst-case laboratory test conditions, Alar 85 SP is classified as 'harmless' to this species.

Remark from previous review: The results are used for risk assessment.

RMS comments and conclusion:

The reported study is GLP compliant and was conducted according to the IOBC/WPRS guideline Heimbach (1992). It is also in line with the current guideline Heimbach *et al.* (2000).

The study results meet the guidelines' validity criteria (mortality in the control treatment over 14 days should not exceed 2 beetles (6.7%): actual value was 0%; corrected mortality in the toxic reference treatment should be 65±35% over 14 days: actual value was 100%).

The study is acceptable for regulatory use.

The 14-day LR₅₀ and ER₅₀ is >10 kg formulation/ha (equivalent to 8.5 kg a.s./ha).

vi) Effects on *Chrysoperla carnea* in laboratory test

Reference:	Barton, R. (1999) A laboratory test to determine the effect of Alar 85 SP (850 g/kg water soluble powder of daminozide) on the green lacewing,
Report No.:	UNI-99-11
Guideline:	IOBC/WPRS Bigler (1988)
GLP:	Yes
Previous evaluation:	Not evaluated in DAR (1999) or Addenda, but included in the Review Report for Daminozide, 2005 (SANCO/3043/99 – Final)
Material and methods:	
Test material:	Alar 85 SP (850 g/kg daminozide)
Lot/Batch No:	SI 6957
Purity:	84.4%

A study to determine the effects of the water-soluble (Alar 85 SP) powder formulation of daminozide (850 g/kg) on the green lacewing, *Chrysoperla carnea* was performed. A group of fifty *C. carnea* larvae were exposed to Alar 85 SP (850 g daminozide/kg) in fifty replicate test units (i.e. exposed individually). Each test unit consisted of a glass plate affixed to a perspex sheet of the same dimensions. A 50mm hole was cut through the top of the Perspex sheet into which an acrylic cylinder (44 mm internal diameter, 20-25 mm tall) was fitted. A fine mesh lid was fitted top the top of the cylinder to prevent the larvae from escaping. The glass plates were treated with Alar 85 SP at a rate equivalent to 10 kg/ha (\cong 8500 g a.s./ha) in a dilution equivalent to 200 L/ha using a Potter laboratory spray tower. Fifty replicate units were also treated with the toxic reference dimethoate at a rate of 102 g a.s./ha in 200 L/ha of water. An additional fifty replicate units treated with tap water at a rate equivalent to 200 L/ha were established as the control.

After allowing ca. 1 hour for drying of the residues on the glass plates, the units were assembled and the larvae introduced along with a surplus of U.V. killed *Sitotroga* eggs to act as a food source, which were replenished every day until pupation. The larvae were examined daily for effects on survival. The test units were placed in a controlled environment (23-27 °C, 60-79% relative humidity and 16 hour photoperiod of 2020-2920 lux). When the larvae had pupated, they were transferred (still attached to the glass plates and acrylic cylinders) into individual ventilated plastic boxes. Within 1-2 days of emergence, adults were counted and transferred to oviposition boxes. Overall percent mortality during the developmental phase was based on larval and pupal mortality combined. During development and maturation, adults were provided with an artificial diet of yeast mixed with honey (1:1)

and made into a paste with water, a 1:2 – 1:3 honey/water solution on a cotton wool pad and fresh water on a cotton wool pad. Environmental conditions during this phase were maintained at 11-26°C, 31-92% RH and 16 hour photoperiod 2020-2240 lux.

Approximately 10 days after the majority of adults had emerged, the fecundity assessments were initiated by placing a thin sheet of fibrous material under the lid of each box to act as a site for oviposition. Two boxes per treatment were established with adult lacewings of a similar sex ratio placed in each box. Effects on fecundity were determined over a 21 day period by making 9 assessments in which numbers of eggs laid per female were recorded. Egg viability was determined by collecting sub-samples of eggs once each week by removing egg sheets and placing in ventilated plastic pots. The numbers of larvae emerging in these pots were counted each day and removed. The sex of adults that died during the test was determined and all surviving adults were sexed at the end of the study.

Abbott formula used to correct for control mortality.

Results:

During the developmental stage of the bioassay, the corrected mortality in the Alar 85 SP group was only 2%, indicating no harmful effects on the larval/pupal stages. In the fecundity assessment, the mean number of viable eggs produced per female per day in the control and Alar 85 SP groups were 15.7% and 15.4% respectively. The mean percentage viability was > 93%. The mean number of viable eggs/female/day was in the control and Alar 85 SP groups were 14.8% and 14.3% respectively.

The results of the test are presented in the table below.

Table B.9.5.2-6 Biological results

The pre-imaginal mortality of the test insects is summarised below.

	Larvae tested	Larvae pupating	Adults emerging	Overall % mortality	Abbott-corrected % mortality
Control	50	47	45	10	-
Alar 85 SP	49	44	43	12	2
Dimethoate	50	0	0	100	100

The results of the fecundity assessments are summarised below.

	Mean No. eggs per female per day	Estimated % viability	Mean No. viable eggs per female per day
Control	15.7	94%	14.8
Alar 85 SP	15.4	93%	14.3

Following exposure to Alar 85 SP at a treatment rate equivalent to 10 kg/ha (\cong 8500 g a.s./ha), larval/pupal mortality of the green lacewing, *Chrysoperla carnea* was 2%. During the fecundity phase, there was no significant difference between eggs laid per female in the control and Alar 85 SP treatment groups. It can therefore be concluded that, under extreme worst-case laboratory test conditions, Alar 85 SP is classified as ‘harmless’ to this species.

Remark from previous review: The results are used for risk assessment.

RMS comments and conclusion:

The reported study is GLP compliant and was conducted according to the IOBC/WPRS guideline Bigler (1988). It is also in line with the current guideline Vogt *et al.* (2000).

The study results meet the guidelines’ validity criteria (mortality in the control treatment over 14 days should not exceed 2 beetles (pre-imaginal mortality in the control group should not exceed 20%: actual mortality was 10%; mortality in the toxic reference treatment should exceed 57.5%: actual value was 100%; mean egg production in the control should be \geq 15 eggs per female per day: actual egg production was 15.7 eggs per female per day; mean hatching rate of the eggs in the control should be \geq 70%: actual egg hatch was 94%).

The study is acceptable for regulatory use.

The 14-day LR₅₀ and ER₅₀ is >10 kg formulation/ha (equivalent to 8.5 kg a.s./ha).

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. (2001a) An extended laboratory test to determine the effect of Alar 85 SP (an 850 g/kg water-soluble powder formulation of daminozide) on the predatory mite <i>Typhlodromus pyri</i> (Acari; Phytoseiidae)
Report No.:	UNI-01-1
Guideline:	IOBC/WPRS Overmeer (1988); Blümel <i>et al.</i> (2000).
GLP:	Yes
Previous evaluation:	In Addendum 1 (2002)

Executive Summary

A study to determine the effects of Alar 85 SP (850 g/kg daminozide) on the predatory mite, *Typhlodromus pyri* was performed. Two application rates were used, one equivalent to 5 kg product/ha and the second one equivalent to 10 kg product/ha. Approximately 1 day-old protonymphs were used for the tests and French bean plants were used as a model crop system. Circular discs were cut from flattened sections of the leaves from the French bean plants and used as test substrates. Five leaf discs per treatment were sprayed on their upper surface before they were placed in the test units. Twenty protonymphal *T. pyri* were placed in the centre of each replicate arena, with five replicates (total 100 mites) per treatment. Survival of the mites was assessed over a 7-day period, by which time they were adult. The sex of the adult mites was determined and they were then left *in situ* so that their fecundity could be assessed over further 7 days. The mean number of eggs produced per female between 7 and 14 days after treatment was calculated. Mean mortality in the control was 19% and 100% in the reference treatment. Mean mortality in the 5 and 10 kg product/ha treatments was 23 and 15%, respectively. After Abbott's correction, mortality was not significantly different from the control. The mean number of eggs produced per female was calculated to be 5.3 in the control treatment, compared with 6 and 5.1 at 5 and 10 kg product/ha, respectively for the Alar 85 SP treatments. Compared to the control this was equivalent to changes in productivity of + 13 and – 4% respectively. Fecundity was not significantly different from the control.

I. MATERIALS AND METHODS**A. MATERIALS**

- 1. Test material:** Alar 85 SP
 - Formulation:** White powder
 - Lot/Batch:** 910M003RJ
 - Purity:** 87.8% w/w
 - Density:** Not stated
- 2. Reference item:** BASF Perfekthion (dimethoate)
 - Formulation:** Emulsifiable concentrate

Lot/Batch: 37M-00217-06
Purity: 400 g/L (nominal), 415.7 g/L (actual)
Density: Not stated

B. STUDY DESIGN AND METHODS

1. Test animals: *Typhlodromus pyri*

Age: 1 day old protonymphs (1-2 days after hatching)

Source: P.K. Nützlingszuchten, Welzheim, Germany)

Acclimation: Not stated

Diet: Walnut and apple pollen

2. Test units: 9 cm plastic Petri dishes lined with cotton wool. Test substrate was leaf discs taken from the French bean *Phaseolus vulgaris* var. The Prince.

3. Environmental conditions:

Temperature: 23-25°C (mortality); 23-26°C (fecundity)

Relative humidity: 57-71% (mortality); 58-75% (fecundity)

Photoperiod: 16 hours light: 8 hours darkness (380 lux)

4. Animal assignment and treatment:

Spray solution was applied to the leaf discs and left to dry. The leaves were then placed on the damp cotton wool in the test units. Five replicate arenas, each containing 20 protonymphs, were prepared for each treatment. Spray solutions were applied to the leaf discs using a Potter Laboratory Spray Tower.

5. Dose preparation:

1.25 g product diluted to 50 mL with water for the application rate of 5 kg product/200 L water/ha. 2.5 g product diluted to 50 mL with water for the application rate of 10 kg product/200 L water/ha.

6. Measurements and observations:

The condition of the mites was examined under a binocular microscope at approximately 24 hours and 7 days after treatment and was recorded as being:

Alive: Still moving

Dead: No sign of movement

Stuck: Embedded in the sticky barrier

Drowned: Dead on the filter paper

Missing: Not visible

For days 7-14 after treatment, total egg production for each arena was determined to calculate egg production per female. Three assessments of oviposition were carried out at 8, 11 and 14 DAT.

7. Statistics:

The number of 'escapees' (i.e. stuck, drowned or missing mites) was added to the number of dead mites in each treatment to derive the overall mortality. The mean percentage mortality was calculated after 7 days and then corrected for any losses in the control treatment using Abbott's formula. A comparison was made of the mortality in the control and test treatments by one-way ANOVA. Prior to analysis, the mortality data were angularly transformed (square root/arcsine) to normalise their distribution.

The mean cumulative number of eggs per female was determined for the period 7-14 DAT. To calculate this value, the total number of eggs laid in each replicate between each assessment date was divided by half of the sum of the numbers of female mites recorded as alive at the start and end of each assessment period. Any progeny recorded as larvae/nymphs were added to the egg totals from the previous assessment period (i.e. the eggs had presumably been missed in a previous assessment and had subsequently hatched). The average for three replicates was calculated and compared by one-way ANOVA.

Results:

A. BIOLOGICAL EFFECTS

The mortality in the control treatment was 19% at 7 DAT compared to 23% and 15% in the 5 and 10 kg product/ha Alar 85 SP treatments, respectively. When adjusted for the control treatment deaths, the corrected mortality was 5% and 0% for the 5 and 10 kg product/ha treatment rates, respectively. 100% mortality was recorded in the reference treatment. There was no significant difference in mortality between the Alar 85 SP treatment and the controls.

The mean number of eggs produced per female was calculated to be 5.3 in the control treatment compared with 6.0 and 5.1 in the 5 and 10 kg product/ha treatments of Alar 85 SP, respectively. When compared to the control, this was equivalent to changes in productivity of +13% and -4%, respectively. The results for the individual treatment did not differ significantly.

A summary of the results is presented in Table 8.3.2.2/03-01 below.

Table B.9.5.2-7 Summary of effects on mortality and fecundity

Treatment	Mortality after 7 days (%)		Fecundity	
	Mortality	Corrected mortality	Mean number of eggs per female	% change relative to control
Control	19	-	5.3	-

Alar 85 SP 5 kg product/ha	23	5	6.0	+13
Alar 85 SP, 10 kg product/ha	15	0	5.1	-4
Dimethoate	100	100	N/A	N/A

B. DEFICIENCIES

None.

Conclusion:

Under the extended laboratory conditions, Alar 85 SP was harmless to the predatory mite, *Typhlodromus pyri*, when applied at rates equivalent to 5 and 10 kg product/ha.

Remark from previous review: Results are used for risk assessment.

RMS comments and conclusion:

The reported study is GLP compliant and was conducted according to the IOBC/WPRS guideline Overmeer (1988) and 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; 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 5.3);).

The study is acceptable for regulatory use.

The 7-day LR₅₀ and the 14-day ER₅₀ is >10 kg formulation/ha (equivalent to 8.5 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> #	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> #	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).

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 Alar.

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

Species	Test substance	Risk quotient	ETR	Trigger
<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 Alar 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	Appl. rate (kg a.s./ha)	Ef	Short-cut value	twa	Endpoint	ETR _{oral}	Trigger	Acceptable risk?
Weeds in the field									
		4.25	0.4 ^a	2.9 µg ^b	0.72		< 0.033	0.03	Yes

Honey bee (adults)	Chronic oral		0.4 ^a	0.27 µg ^c	0.72	> 106.2 µg/bee	< 0.003	0.03	Yes
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^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
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

Species	Test substance	Risk quotient	ETR	Trigger
<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 rhopalosiphii</i>	Adult	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, 4}	Dazide 85	Tier I Glass plate Limit test	Control 10 form. (8.5 a.s.)	n.a.	n.a.	Harwood 2000; 18133
	Protonymph	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	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-00-0
<i>Orius laevigatus</i>	Adult	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	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	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	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 ⁴	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 Dazide Enhance

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 Alar. Therefore, endpoints derived from all the studies on non-target arthropods can be used for the risk assessment for Alar.

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

The product Alar 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 for non-target arthropods, however, for protected use other than permanent greenhouses, the risk assessment for for non-target arthropods assuming the same exposure as for a field 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 (Alar) 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

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).

Overall, a low risk to non-target arthropods can be concluded for the proposed field use of Alar 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 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.9.8.1-1 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). Alar 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 Alar 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 Alar.

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

i) Effects on soil nitrogen transformations

Reference:	Maas, G. (1987, 1989) Effects on soil non-target organisms
Report No.:	A.8.1.18
Guideline:	BBA VI 1-1
GLP:	No
Previous evaluation:	In DAR (1999)

Executive Summary

Daminozide was applied as Alar 85 at test concentrations of 5.33 or 26.7 mg/kg soil. Soils that had not been treated with pesticides in the previous year and had been acclimatised for three weeks at 20°C and 50% MHC were tested in triplicates. Soil samples were incubated at 60% MWHC. For the nitrification test ammonium sulphate was added. After 28 days the effects on nitrification and respiration were <25%. However, in the nitrification test, all nitrogen consisted of NO₂ and NO₃ at all-time points including t = 0 (almost no NH₄), including the comparison (positive) controls (Triphenylformazan (TPF)). The (lack of) influence on ammonium conversion was therefore not demonstrated in the two soils.

Materials and methods:

A. MATERIALS

1. **Test material:** Alar 85
Description: Not stated
Lot/Batch: Not stated
Purity: 85% w/w

B. STUDY DESIGN AND METHODS

1. **Test soil:** Soils without pesticide treatment in the previous year
Source: Not stated
Type: Clay 6.9%, silt 39%, sand 54.1% (soil 1); clay 29.5%, silt 35.9%, sand 34.6% (soil 2)
pH: 6.8 (soil 1); 7.1 (soil 2)
Total organic carbon: 0.95% (soil 1); 2.42% (soil 2)
Microbial biomass: 132.9 mg (soil 1); 522.7 mg (soil 2)
2. **Test vessels:** Not stated
3. **Environmental conditions:**
Temperature: 20°C
pH of soil: Nitrogen turnover: 1.728 (soil 1); 6.473 (soil 2)

4. Preparation of soil:

Soils were incubated at 60% MWHC and had been acclimatised for three weeks at 20°C and 50% MHC.

5. Dose preparation:

Test material was applied at 5.33 and 26.7 mg/kg of soil. Ammonium sulphate was added in the nitrification test.

For comparison, Triphenylformazan (TPF) was tested using the two soils.

6. Measurements and analysis:

Three replicates were used for each test. Respiration was measured after 14 and 28 days. Nitrification was measured at 7, 14, 21 and 28 days.

Results:

A. NITRIFICATION

Effects on nitrification were <25%.

B. SHORT-TERM RESPIRATION

Effects on short-term respiration were <25%.

A summary of the results is presented in Tables 8.5/01-01 and 8.5/01-02 below.

Table B.9.9-1 Summary of effects on nitrification and short-term respiration (soil 1)

Test item mg a.s./kg soil dw	A = NH_4^+ -N levels (day 28)	B = NO_2^- - NO_3^- - N (day 28)		A+B (day 28)		Respiration rate (day 28)	
	mg/kg soil dw	mg/kg soil dw/d	% deviation from control	mg/kg soil dw/d	% deviation from control	mg CO_2 / 100 g soil dw	% deviation from control
Control	0.066 ± 0.030	11.311 ± 0.0149	-	11.377 ± 0.0119	-	2.253 ± 0.085	-
5.33	0.046 ±0.030	11.178 ± 0.259	-1.18	11.225 ± 0.289	1.34	2.207 ± 0.198	-2.05
26.7	0.073 ± 0.011	11.489 ± 0.188	-1.57	11.562 ± 0.188	-1.63	2.345 ± 0.162	+4.1
Positive control (5.33)	0.059 ± 0.020	11.027 ± 0.050	2.51	11.086 ± 0.046	2.56	0.913 ± 0.090	-59.45
Positive control (26.7)	8.483 ± 0.378	2.273 ± 0.109	79.9	10.756 ± 0.394	5.46	0.060 ± 0.105	-73.12

Table B.9.9-2 Summary of effects on nitrification and short-term respiration (soil 2)

Test item mg a.s./kg soil dw	A = NH ⁴⁺ -N levels (day 28)	B = NO ₂ ⁻ - NO ₃ ⁻ - N (day 28)		A+B (day 28)		Respiration rate (day 28)	
	mg/kg soil dw	mg/kg soil dw/d	% deviation from solvent control	mg/kg soil dw/d	% deviation from solvent control	mg CO ₂ /100 g soil dw	% deviation from solvent control
Control	0.091 ± 0.088	15.724 ± 0.193	-	15.816 ± 0.129	-	5.88 ± 0.274	-
5.33	0.225 ± 0.074	16.145 ± 0.220	-2.68	16.370 ± 0.231	-3.50	6.561 ± 0.222	+11.59
26.7	0.119 ± 0.044	15.549 ± 0.196	1.011	15.668 ± 0.159	0.94	5.874 ± 0.227	-0.09
Positive control (5.33)	0.032 ± 0.021	15.759 ± 0.377	-0.22	15.791 ± 0.360	0.16	5.209 ± 0.291	-11.40
Positive control (26.7)	0.091 ± 0.012	15.661 ± 0.950	0.40	15.725 ± 0.939	0.57	1.956 ± 0.057	-66.73

B. DEFICIENCIES

None.

Conclusion:

Based on the results of this study and when considered in accordance with the OECD Guidelines 216 and 217, the test item had no adverse effect on soil respiration and nitrogen turnover (day 28) in an agricultural soil tested up to 26.7 mg a.s./kg soil dry weight at study termination.

Remark from previous review: The effects (E) can be classified based on the BBA Guidelines: class 1 (= negligible). For risk evaluation the following results are used: no persistent effect on soil respiration (two soils) and soil nitrification (one soil).

RMS comments and conclusion:

The reported study is non-GLP and was conducted according to BBA guideline VI 1-1 (1990), which is in line with the current OECD 216 (2000) and OECD 217 (2000) guidelines. However, the quantity of nitrate formed in each replicate soil sample should be recorded according to OECD 216 TG while mean values were only reported in the study. Therefore, it was not possible to check the guidelines' validity criteria (the variation between replicate control samples should be less than ± 15%).

The study is not considered valid.

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 - Alar 85 WSG**

Reference:	Sindermann, A.B., Porch, J.R., Krueger, H.O. and Martin, K.H. (2012b) Daminozide formulation: A toxicity test to determine the effects (Tier II) on vegetative vigor of ten species of plants
Report No.:	616-108
Guideline:	OECD 227 (2006); OPPTS 850.4250 (1996)
GLP:	Yes
Previous evaluation:	Submitted for the purpose of renewal

Executive Summary:

The effects of post-emergence exposure of daminozide formulation (Alar 85 WSG) on vegetative vigour of ten species of terrestrial plant were tested in a laboratory study. Seedlings were exposed to the test item concentrations of 469, 938, 1875, 3750 and 7500 ppm applied at nominal rates of 2037 litres of solution per hectare, plus a negative control was tested in parallel.

The results demonstrated no adverse effects on the height, survival, dry weight and plant condition in onion, ryegrass, wheat and corn (NOER = 7500 ppm). For sugarbeet, effects on dry weight were observed resulting in a NOER of 938 ppm. Based on effects on height the NOER for cabbage, tomato, lettuce, oilseed rape and soybean were determined to be <469, 938, 469, 938 and 469, respectively. Since a treatment-group reduction of 25% or greater relative to control means was not observed, the ER₂₅ and ER₅₀ for all parameters were greater than 7500 ppm.

Materials and methods:

A. MATERIALS

1. **Test material:** Alar 85 WSG (B-Nine)
Description: Solid
Lot/Batch: BO0J15P033
Purity: 85.5% (w/w) (active substance: daminozide)

B. STUDY DESIGN AND METHODS

1. Test species:

Species	Common name	Variety	Family
Monocots:			
<i>Allium cepa</i>	Onion	Yellow Granex Hybrid 33	Liliaceae
<i>Lolium perenne</i>	Ryegrass	Gator 3 Perennial	Poaceae
<i>Triticum aestivum</i>	Wheat	Glenn Hard Red Spring	Poaceae
<i>Zea mays</i>	Corn	Jarvis Golden Prolific	Poaceae
Dicots:			
<i>Beta vulgaris</i>	Sugarbeet	Beta4609R	Chenopodiaceae
<i>Brassica napus</i>	Oilseed rape	Dwarf Essex	Brassicaceae
<i>Brassica oleracea</i>	Cabbage	Late Flat Dutch	Brassicaceae
<i>Glycine max</i>	Soybean	Maverick	Fabaceae
<i>Lactuca sativa</i>	Lettuce	Summertime	Asteraceae
<i>Lycopersicon esculentum</i>	Tomato	Rutgers	Solanaceae

2. **Test units:** Plastic pots (16 cm in diameter and 12 cm deep)
Test soil: Loamy sand soil (with added slow release fertiliser)

Composition: 85% sand, 5% silt and 12% clay, with an organic carbon content of 1.2%

pH: 5.7

3. Treatment groups: 0, 469, 938, 1875, 3750 and 7500 ppm

4. Environmental conditions:

Temperature: 14.9 – 32.2°C

Relative humidity: 9.2 – 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 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 six replicated experimental units consisting of five plants, 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 spray mixtures for the highest treatment group, spray solutions with nominal concentrations of 7500 ppm (6413 ppm a.s.), were prepared by diluting 15.0003 and 15.0000 g of the test substance, respectively, to final volumes of two litres in a volumetric flask with water purified by reverse osmosis. Solutions were mixed by swirling, sonication and inversion. Test substance spray mixtures were observed to be clear liquids. Spray mixtures for subsequent treatment groups were prepared using proportional volumetric dilutions. Diluting aliquots of 62.5, 125, 250 and 500 mL each to final volumes of 1000 mL with water purified by reverse osmosis resulted in spray mixtures for the 469, 938, 1875 and 3750 ppm treatment groups, respectively. Negative control spray was water purified by reverse osmosis. New spray mixtures were prepared on each day of test substance application and applied only on the day they were prepared.

Application of the test substance or negative control spray mixture was made to the soil surface of planted pots using a calibrated DeVries Research Track Sprayer (spray booth). Spray mixtures were applied at a nominal spray volume of 2037 litres per hectare (L/ha).

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.

Spray mixtures were sampled following preparation for confirmation of their test substance concentrations. Triplicate samples were collected from the spray mixtures with the lowest and highest test substance concentration. Single samples were collected from the negative control spray mixture, and spray mixtures for intermediate concentrations of the test substance. Concentrations of daminozide in diluents of the samples were determined by high performance liquid chromatography using an Agilent Series 1100 High Performance Liquid Chromatograph (HPLC) coupled with an Applied Biosystems Sciex API 3000 LC Mass Spectrometer (MS/MS).

The light intensity, temperature, and relative humidity within the greenhouse were continuously monitored during the test with a data-logger.

8. Statistics:

Treatment group means were compared to negative control means using the Jonckheere-Terpstra Test for Trend, ($\alpha = 0.05$). Where reductions in treatment group means were 25% or greater relative to the control means, effect rates (ER estimates) estimating the rates producing a 25% reduction and their confidence limits were determined using the non-linear regression analysis of Bruce and Versteeg.

Results:

A. BIOLOGICAL EFFECTS:

There were no adverse treatment-related effects on the height, weight, or condition of the four monocot species tested. In each of the six dicot species tested, there were statistically significant ($p < 0.05$) reductions of mean height in comparison to the respective control means. With the exception of *G. max* (soybean), all treatment group reductions were less than 25% in magnitude.

There were no adverse effects from treatment on the dry weight of *B. napus* (oilseed rape) and *B. oleracea* (cabbage). The mean dry weight of *B. vulgaris* (sugarbeet), *G. max* (soybean), *L. sativa* (lettuce) and *L. esculentum* (tomato) were significantly different ($p < 0.05$) from the respective control means.

There were no adverse effects of treatment on the survival of the ten species tested. One *A. cepa* plant in the 1875 ppm treatment group died, but this mortality appeared to be incidental.

Visual signs of phytotoxicity observed on test plants included chlorosis, necrosis, and leaf curl. In general signs were categorized as slight, although a few seedlings were given scores for more severe conditions. Signs were more prevalent on plants of higher treatment groups of all species, with the exception of *B. oleracea*, and dose-responsive signs were considered to be effects of the daminozide formulation. Three replicates inadvertently did not receive sufficient water (Replicate C of the 7500 ppm treatment group of *B. napus*, and Replicate E of the

Negative Control and 3750 ppm treatment groups of *B. oleracea*), and plants were therefore removed from statistical analyses. In most cases, signs of toxicity were reduced in intensity by Day 21, however in *G. max* (soybean) signs of toxicity generally increased in severity over the course of the 21 day study.

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

Table B.9.9.1-1 Effects of daminozide formulation on mean height, dry weight and survival on day 21 of the vegetative vigour test

Species	Treatment group	Height (cm) (% reduction)	Dry weight (mg) (% reduction)	% Survival (% reduction)
Onion <i>Allium cepa</i>	Control	30.1	0.249	100.0
	469 ppm	32.7 (-9)	0.301 (-21)	100.0 (0)
	938 ppm	29.9 (1)	0.238 (4)	100.0 (0)
	1875 ppm	30.2 (0)	0.239 (4)	96.7 (3)
	3750 ppm	30.2 (0)	0.280 (-13)	100.0 (0)
	7500 ppm	30.3 (-1)	0.255 (-2)	100.0 (0)
Ryegrass <i>Lolium perenne</i>	Control	26.4	1.009	100.0
	469 ppm	25.9 (2)	0.962 (5)	100.0 (0)
	938 ppm	25.8 (2)	0.946 (6)	100.0 (0)
	1875 ppm	26.9 (-2)	0.933 (7)	100.0 (0)
	3750 ppm	25.0 (5)	0.927 (8)	100.0 (0)
	7500 ppm	25.8 (2)	0.956 (5)	100.0 (0)
Wheat <i>Triticum aestivum</i>	Control	52.5	0.985	100.0
	469 ppm	50.9 (3)	0.926 (6)	100.0 (0)
	938 ppm	51.0 (3)	0.875 (11)	100.0 (0)
	1875 ppm	51.3 (2)	0.911 (7)	100.0 (0)
	3750 ppm	52.2 (1)	0.937 (5)	100.0 (0)
	7500 ppm	50.9 (3)	0.910 (8)	100.0 (0)
Corn <i>Zea mays</i>	Control	66.0	1.81	100.0
	469 ppm	70.0 (-6)	2.04 (-13)	100.0 (0)
	938 ppm	63.4 (4)	1.58 (12)	100.0 (0)
	1875 ppm	64.2 (3)	1.69 (6)	100.0 (0)
	3750 ppm	68.9 (-4)	1.94 (-7)	100.0 (0)
	7500 ppm	66.6 (-1)	1.79 (1)	100.0 (0)
Sugarbeet <i>Beta vulgaris</i>	Control	17.2	1.56	100.0
	469 ppm	15.7 (9)	1.40 (10)	100.0 (0)
	938 ppm	17.2 (0)	1.49 (4)	100.0 (0)
	1875 ppm	15.6 (9)	1.32 (15)*	100.0 (0)

Species	Treatment group	Height (cm) (% reduction)	Dry weight (mg) (% reduction)	% Survival (% reduction)
	3750 ppm	15.7 (9)*	1.26 (19)*	100.0 (0)
	7500 ppm	15.7 (9)*	1.30 (17)*	100.0 (0)
Oilseed rape <i>Brassica napus</i>	Control	23.7	3.19	100.0
	469 ppm	22.7 (4)	3.10 (3)	100.0 (0)
	938 ppm	22.1 (7)	3.02 (5)	100.0 (0)
	1875 ppm	20.3 (14)*	3.08 (3)	100.0 (0)
	3750 ppm	19.9 (16)*	3.15 (1)	100.0 (0)
	7500 ppm	19.8 (16)*	2.89 (9)	100.0 (0)
Cabbage <i>Brassica oleracea</i>	Control	20.9	3.07	100.0
	469 ppm	18.3 (13)*	2.477 (10)	100.0 (0)
	938 ppm	19.0 (9)*	2.66 (13)	100.0 (0)
	1875 ppm	17.8 (15)*	2.83 (8)	100.0 (0)
	3750 ppm	18.3 (13)*	2.76 (10)	100.0 (0)
	7500 ppm	17.9 (15)*	2.81 (8)	100.0 (0)
Soybean <i>Glycine max</i>	Control	28.6	2.55	100.0
	469 ppm	26.4 (8)	2.40 (6)	100.0 (0)
	938 ppm	26.0 (9)*	2.53 (1)	100.0 (0)
	1875 ppm	23.9 (17)*	2.40 (6)	100.0 (0)
	3750 ppm	22.1 (23)*	2.37 (7)	100.0 (0)
	7500 ppm	20.4 (29)*	2.27 (11)*	100.0 (0)
Lettuce <i>Lactuca sativa</i>	Control	11.3	2.95	100.0
	469 ppm	11.1 (2)	2.89 (2)	100.0 (0)
	938 ppm	10.0 (12)*	2.50 (15)	100.0 (0)
	1875 ppm	10.6 (6)*	2.71 (8)	100.0 (0)
	3750 ppm	10.0 (11)*	2.75 (7)	100.0 (0)
	7500 ppm	9.7 (14)*	2.53 (14)*	100.0 (0)
Tomato <i>Lycopersicon esculentum</i>	Control	28.4	3.18	100.0
	469 ppm	28.0 (1)	3.07 (3)	100.0 (0)
	938 ppm	28.2 (1)	3.25 (-2)	100.0 (0)
	1875 ppm	26.6 (6)	2.92 (8)	100.0 (0)
	3750 ppm	25.9 (9)*	2.78 (13)	100.0 (0)
	7500 ppm	24.6 (13)*	2.67 (16)	100.0 (0)

* Treatment group mean was significantly different from the control mean ($p < 0.05$) using the Jonckheere-Terpstra Test for Trend.

The effects of test substance application on the height and dry weight of each of the test species 21 days after application expressed in terms of the NOER, LOER, ER₂₅ and ER₅₀ are presented in the tables below.

Table B.9.9.1-2 21-day toxicity of daminozide formulation on vegetative vigour based on height and dry weight

Species	Endpoint	Effect rates for height	Effect rates on dry weight
		Value (ppm)	
Onion <i>Allium cepa</i>	NOER	7500	7500
	LOER	>7500	>7500
	ER ₂₅	>7500	>7500
	ER ₅₀	>7500	>7500
Ryegrass <i>Lolium perenne</i>	NOER	7500	7500
	LOER	>7500	>7500
	ER ₂₅	>7500	>7500
	ER ₅₀	>7500	>7500
Wheat <i>Triticum aestivum</i>	NOER	7500	7500
	LOER	>7500	>7500
	ER ₂₅	>7500	>7500
	ER ₅₀	>7500	>7500
Corn <i>Zea mays</i>	NOER	7500	7500
	LOER	>7500	>7500
	ER ₂₅	>7500	>7500
	ER ₅₀	>7500	>7500
Sugarbeet <i>Beta vulgaris</i>	NOER	1875	938
	LOER	3750	1875
	ER ₂₅	>7500	>7500
	ER ₅₀	>7500	>7500
Oilseed rape <i>Brassica napus</i>	NOER	938	7500
	LOER	1875	>7500
	ER ₂₅	>7500	>7500
	ER ₅₀	>7500	>7500
Cabbage <i>Brassica oleracea</i>	NOER	<469	7500
	LOER	469	>7500
	ER ₂₅	>7500	>7500
	ER ₅₀	>7500	>7500
Soybean <i>Glycine max</i>	NOER	469	3750
	LOER	938	7500
	ER ₂₅	5036	>7500
	ER ₅₀	>7500	>7500
Lettuce	NOER	469	3750

Species	Endpoint	Effect rates for height	Effect rates on dry weight
		Value (ppm)	
<i>Lactuca sativa</i>	LOER	938	7500
	ER ₂₅	>7500	>7500
	ER ₅₀	>7500	>7500
Tomato <i>Lycopersicon esculentum</i>	NOER	938	3750
	LOER	1875 ^a	7500
	ER ₂₅	>7500	>7500
	ER ₅₀	>7500	>7500

^a The value was not statistically significantly different from the control but was considered to be an effect level.

B. ANALYSIS:

Samples collected to demonstrate homogeneity and verify test substance concentrations for the 401 and 6413 ppm a.s. spray mixture had a mean and standard deviation of 387 ± 37.0 and 6588 ± 64.4 ppm a.s., respectively. These values represented 97% and 103% of the nominal concentration.

Samples collected to verify test substance concentrations are summarised in the tables below.

Table B.9.9.1-3 Analytical determination of daminozide in spray solution

Sample date	Spray mixture concentration (ppm a.s.)		
	Nominal	Measured ^{a,b}	Percent of nominal
January 26, 2012	0	<LOQ	--
	401	407	101
	401	409	102
	401	344	86
	802	804	100
	1603	1578	98
	3206	3291	103
	6413	6627	103
	6413	6514	102
	6413	6624	103
January 27, 2012	0	<LOQ	--
	401	371	93
	401	397	99
	401	411	102
	802	760	95
	1603	1538	96

Sample date	Spray mixture concentration (ppm a.s.)		
	Nominal	Measured ^{a,b}	Percent of nominal
	3206	3250	101
	6413	6490	101
	6413	6289	98
	6413	6377	99

^a Measured concentration in analytical samples collected from spray mixtures.

^b The method limit of quantitation (LOQ) for the analysis of daminozide was set at 25 mg a.s./L, calculated as the product of the concentration of the lowest calibration standard (0.0500 mg a.s./L) and the dilution factor of the matrix blank sample (500).

Conclusion

A foliar application of daminozide formulation resulted in no adverse effects on the height, survival, dry weight and plant condition in onion, ryegrass, wheat and corn (NOER = 7500 ppm). For sugarbeet, effects on dry weight were observed resulting in a NOER of 938 ppm. Based on effects on height the NOER for cabbage, tomato, lettuce, oilseed rape and soybean were determined to be <469, 938, 469, 938 and 469, respectively. Since a treatment-group reduction of 25% or greater relative to control means was not observed, the ER₂₅ and ER₅₀ for all parameters were greater than 7500 ppm.

RMS comments and conclusion:

The reported study is GLP compliant and was conducted according to OECD 227 (2006) and OPPTS 850.4250 (1996) guidelines. The test results are in compliance with the guidelines' validity criteria: there was more than 90% survival in the control plants (actual value was 100%); the environmental conditions were identical for all the tested species; the control plants did not exhibit any phytotoxic effects – except for slight chlorosis and necrosis in 1 replicate of maize, slight leaf curl in 1 replicate of sugar be (recovered by day 21) and slight to moderate wilt and necrosis in one replicate of cabbage (recovered by day 21). The study is still acceptable for regulatory use.

The ER₅₀ for all parameters were greater than than 7500 ppm formulation, equivalent to 6413 ppm a.s. which is equivalent to 13 kg a.s./ha, the highest concentration tested.

ii) A seedling emergence test - Alar 85 WSG

Reference:	Sindermann, A.B., Porch, J.R., Krueger, H.O. and Martin, K.H. (2012a) Daminozide formulation: A toxicity test to determine the effects (Tier II) on seedling emergence of ten species of plants
Report No.:	616-107

Guideline: OECD 208 (2006); OPPTS 850.4225 (1996)

GLP: Yes

Previous evaluation: Submitted for the purpose of renewal

Executive Summary:

The effects of pre-emergence exposure of daminozide formulation (Alar 85 WSG) on seedling emergence of ten species of terrestrial plant were tested in a laboratory study. Seeds were exposed to the test item concentrations of 469, 938, 1875, 3750 and 7500 ppm applied at nominal rates of 2037 litres of solution per hectare, plus a negative control was tested in parallel.

The results demonstrated no adverse effects on the emergence, survival, height, dry weight and plant condition of all species tested. Therefore, the NOER was the highest test level, a test substance concentration of 7500 ppm, and the LOER was greater than 7500 ppm. Since a treatment-group reduction of 25% or greater relative to control means was not observed, the ER₂₅ and ER₅₀ for all parameters were greater than 7500 ppm.

Materials and methods:

A. MATERIALS

1. **Test material:** Alar 85 WSG (B-Nine)
Description: Solid
Lot/Batch: BO0J15P033
Purity: 85.5% (w/w) (active substance: daminozide)

B. STUDY DESIGN AND METHODS

1. Test species:

Species	Common name	Variety	Family
Monocots:			
<i>Allium cepa</i>	Onion	Yellow Granex Hybrid 33	Liliaceae
<i>Lolium perenne</i>	Ryegrass	Gator 3 Perennial	Poaceae
<i>Triticum aestivum</i>	Wheat	Glenn Hard Red Spring	Poaceae
<i>Zea mays</i>	Corn	Jarvis Golden Prolific	Poaceae
Dicots:			
<i>Beta vulgaris</i>	Sugarbeet	Beta4609R	Chenopodiaceae
<i>Brassica napus</i>	Oilseed rape	Dwarf Essex	Brassicaceae
<i>Brassica oleracea</i>	Cabbage	Late Flat Dutch	Brassicaceae
<i>Glycine max</i>	Soybean	Maverick	Fabaceae
<i>Lactuca sativa</i>	Lettuce	Summertime	Asteraceae

Lycopersicon esculentum Tomato

Rutgers

Solanaceae

2. **Treatment groups:** 0, 469, 938, 1875, 3750 and 7500 ppm

3. **Test units:** Plastic pots (16 cm in diameter and 12 cm deep)

Test soil: Loamy sand soil (with added slow release fertiliser)

Composition: 85% sand, 5% silt and 12% clay, with an organic carbon content of 1.2%

pH: 5.7

4. **Environmental conditions:**

Temperature: 17.2 – 31.9°C

Relative humidity: 10.5 – 75.5%

Photoperiod: 16 hours light: 8 hours darkness

5. **Organism assignment and treatment:**

The experimental design consisted of five treatment groups and a control group for each test species. Within test groups there were four replicated experimental units consisting of a pot with ten planted seeds. A single application of the test substance was made to the soil surface of pots in respective treatment groups. A single application of water purified by reverse osmosis was made to the soil surface of pots in the negative control group. Spray solutions were applied at a nominal spray volume of 2037 litres per hectare (L/ha), approximately 200 gallons per acre. In addition to the negative control, test substance concentrations of 469, 938, 1875, 3750, and 7500 parts of test substance per million of solution (ppm) were evaluated on each species. After application, planted pots were placed in sub-irrigation trays on benches in the greenhouse. The replicates were arranged according to a randomised block design. Test duration was 21 days.

6. **Dose preparation:**

Stock solutions with nominal test substance concentrations of 6413 ppm a.s. (7500 ppm) were prepared by diluting 3.7500 g of the test substance to a final volume of 500 mL with water purified by reverse osmosis and mixing by swirling, inversion, and sonication. The physical appearances of the stock solutions were described as clear liquids. The stock solutions served as the spray mixture for the highest application rate. Spray mixtures for the remaining treatment groups were prepared by proportional dilution of the stock solutions. A negative control spray mixture consisted of water purified by reverse osmosis. New spray mixtures were prepared on each day of application, and spray mixtures were only used on the day they were prepared.

Application of the test substance or negative control spray mixture was made to the soil surface of planted pots using a calibrated DeVries Research Track Sprayer (spray booth). Spray mixtures were applied at a nominal spray volume of 2027 or 2050 litres per hectare (L/ha).

7. **Measurements and observations:**

Observations for seedling emergence were conducted on 7, 14, and 21 days after application. On test termination, height measurements and seedling conditional assessments were made. After the final plant heights and visual assessments were made, plant shoots were collected, dried, and weighed. Living seedlings at test termination were collected, dried, and weighed by replicate after final observations.

Spray mixtures were sampled following preparation for confirmation of their test substance concentrations. Triplicate samples were collected from the spray mixtures with the lowest and highest test substance concentration. Single samples were collected from the negative control spray mixture, and spray mixtures for intermediate concentrations of the test substance. Concentrations of daminozide in diluents of the samples were determined by high performance liquid chromatography using an Agilent Series 1100 High Performance Liquid Chromatograph (HPLC) coupled with an Applied Biosystems Sciex API 3000 LC Mass Spectrometer (MS/MS).

The light intensity, temperature, and relative humidity within the greenhouse were continuously monitored during the test with a data-logger.

8. Statistics:

Treatment group means were compared to negative control means using a Dunnett's t-test ($\alpha = 0.05$). Supporting statistics, such as those testing normal distribution, and homogeneity of variance, as well as an analysis of variance were performed on the data.

Results:

A. BIOLOGICAL EFFECTS:

There were no adverse effects on the emergence, survival, height, dry weight, and plant condition of the species tested. No treatment group means were significantly different ($p > 0.05$) from the respective control mean.

The 469 ppm treatment group dry weight mean of *B. napus*, the 1875 ppm treatment group dry weight mean of *B. oleracea*, and the 938 ppm treatment group emergence mean of *B. oleracea* were reduced by 16, 17, and 16% from the control means, respectively. These reductions were not dose-responsive, and the treatment group means were not significantly different ($p > 0.05$) compared to the control means. The 7500 ppm treatment group dry weight means of *B. oleracea* and *L. sativa* were reduced by 16 and 22% from control means, respectively. However, these replicates means overlapped with control values, and the treatment group means were not significantly different ($p > 0.05$) from the control mean. Therefore, these reductions were not considered adverse effects of treatment.

A few seedlings in the test exhibited signs of phytotoxicity, such as chlorosis, mortality, necrosis, and leaf curl. Scores rating the effect of individual plant conditions varied from slight to severe. However, overall, mean replicate scores were slight, and signs were not present on most seedlings in a replicate and treatment group.

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

Table B.9.9.1-4 Effects of daminozide formulation on mean emergence, survival, height and dry weight on day 21 of the seedling emergence test

Species	Treatment group	% Emergence (% reduction)	% Survival (% reduction)	Dry weight (mg) (% reduction)	Height (cm) (% reduction)
Onion <i>Allium cepa</i>	Control	9.75	95.0	7.91	8.8
	469 ppm	10.00 (-3)	100.0 (-5)	8.71 (-10)	9.6 (-9)
	938 ppm	10.00 (-3)	100.0 (-5)	7.73 (2)	8.9 (0)
	1875 ppm	10.00 (-3)	100.0 (-5)	7.91 (0)	8.9 (0)
	3750 ppm	9.75 (0)	100.0 (-5)	8.10 (-2)	9.3 (-5)
	7500 ppm	9.75 (0)	100.0 (-5)	8.06 (-2)	8.5 (3)
Ryegrass <i>Lolium perenne</i>	Control	9.5	100.0	12.5	12.6
	469 ppm	8.75 (8)	100.0 (0)	12.2 (2)	12.6 (0)
	938 ppm	9.75 (-3)	97.5 (3)	15.3 (-23)	14.2 (-13)
	1875 ppm	9.75(-3)	100.0 (0)	14.0 (-13)	13.5 (-7)
	3750 ppm	9.25 (3)	100.0 (0)	15.2 (-22)	13.8 (-10)
	7500 ppm	9.25(3)	97.2 (3)	12.7 (-2)	12.6 (0)
Wheat <i>Triticum aestivum</i>	Control	10.00	100.0	160	40.7
	469 ppm	9.50 (5)	100.0 (0)	157 (2)	39.7 (2)
	938 ppm	9.75 (3)	100.0 (0)	175 (-9)	41.0 (-1)
	1875 ppm	9.00 (10)	96.9 (3)	152 (6)	39.1 (4)
	3750 ppm	9.75 (3)	97.5 (3)	159 (1)	41.1 (-1)
	7500 ppm	9.50 (5)	100.0 (0)	151 (6)	40.0 (2)
Corn <i>Zea mays</i>	Control	9.25	100.0	325	39.8
	469 ppm	10.00 (-8)	97.5 (3)	336 (-4)	40.9 (-3)
	938 ppm	9.50 (-3)	100.0 (0)	339 (-5)	42.0 (-6)
	1875 ppm	9.50 (-3)	100.0 (0)	330 (-2)	40.5 (-2)
	3750 ppm	9.75 (-5)	100.0 (0)	293 (10)	39.5 (1)
	7500 ppm	10.00 (-8)	100.0 (0)	304 (6)	36.9 (7)
Sugarbeet <i>Beta vulgaris</i>	Control	8.25	100.0	99.9	12.6
	469 ppm	8.75 (-6)	100.0 (0)	87.8 (12)	11.9 (6)
	938 ppm	8.00 (3)	97.2 (3)	101.3 (-3)	12.8 (-1)
	1875 ppm	8.25 (0)	100.0 (0)	103.1 (-3)	13.2 (-5)
	3750 ppm	8.75 (-6)	97.2 (3)	91.9 (8)	11.9 (6)
	7500 ppm	8.25 (0)	100.0 (0)	99.2 (1)	12.2 (3)
Oilseed rape	Control	9.00	97.5	311	19.9

Species	Treatment group	% Emergence (% reduction)	% Survival (% reduction)	Dry weight (mg) (% reduction)	Height (cm) (% reduction)
<i>Brassica napus</i>	469 ppm	8.75 (3)	91.9 (6)	263 (16)	18.5 (7)
	938 ppm	9.50 (-6)	100.0 (-3)	298 (4)	19.4 (2)
	1875 ppm	9.75 (-8)	97.5 (0)	276 (11)	18.8 (5)
	3750 ppm	9.75 (-8)	100.0 (-3)	266 (14)	19.2 (4)
	7500 ppm	9.50 (-6)	100.0 (-3)	277 (11)	17.4 (13)
Cabbage <i>Brassica oleracea</i>	Control	9.25	100.0	143	15.5
	469 ppm	8.75 (5)	91.0 (9)	153 (-7)	15.9 (-3)
	938 ppm	7.75 (16)	100.0 (0)	127 (11)	14.7 (5)
	1875 ppm	9.00 (3)	97.5 (3)	119 (17)	14.6 (6)
	3750 ppm	9.00 (3)	94.7 (5)	126 (11)	14.9 (4)
	7500 ppm	8.50 (8)	100.0 (0)	120 (16)	14.4 (7)
Soybean <i>Glycine max</i>	Control	9.75	100.0	378	20.6
	469 ppm	10.00 (-3)	97.5 (3)	352 (7)	18.5 (10)
	938 ppm	9.75 (0)	100.0 (0)	358 (5)	19.4 (6)
	1875 ppm	10.0 (-3)	97.5 (3)	351 (7)	19.9 (3)
	3750 ppm	10.0 (-3)	100.0 (0)	356 (6)	19.4 (6)
	7500 ppm	9.75 (0)	100.0 (0)	349 (8)	18.2 (11)
Lettuce <i>Lactuca sativa</i>	Control	9.75	100.0	56.3	10.3
	469 ppm	9.50 (3)	96.9 (3)	53.0 (6)	9.3 (9)
	938 ppm	9.50 (3)	100.0 (0)	49.8 (12)	9.8 (4)
	1875 ppm	9.25 (5)	100.0 (0)	58.4 (-4)	10.6 (-3)
	3750 ppm	9.50 (3)	100.0 (0)	52.9 (6)	10.0 (3)
	7500 ppm	9.25 (5)	97.5 (3)	43.9 (22)	9.1 (12)
Tomato <i>Lycopersicon esculentum</i>	Control	9.75	100.0	71.1	9.3
	469 ppm	8.75 (10)	100.0 (0)	66.7 (6)	9.1 (2)
	938 ppm	9.50 (3)	100.0 (0)	79.4 (-12)	10.2 (-11)
	1875 ppm	9.25 (5)	100.0 (0)	83.7 (-18)	11.1 (-19)
	3750 ppm	9.25 (5)	100.0 (0)	63.3 (11)	9.3 (0)
	7500 ppm	10.00 (-3)	100.0 (0)	62.3 (13)	9.1 (2)

Given the absence of adverse effects observed in the emergence, survival, height, and dry weight of the ten species tested, the NOER was determined to be 7500 ppm, the highest test solution concentration, and the LOER was greater than the highest test solution concentration. Since a treatment group reduction of 25% or greater

relative to control means was not observed in any of the measured parameters of the tens species, the ER₂₅ and ER₅₀ was greater than 7500 ppm, the highest test solution.

B. ANALYSIS:

Samples collected to demonstrate homogeneity and verify test substance concentrations for the 401 and 6413 ppm a.s. spray mixture had a mean and standard deviation of 391 ± 86.2 and 6389 ± 44.0 ppm a.s., respectively. These values represented 98% and 100% of the nominal concentration.

Samples collected to verify test substance concentrations are summaries in the tables below.

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

Sample date	Spray mixture concentration (ppm a.s.)		
	Nominal	Measured ^{a,b}	Percent of nominal
January 16, 2012	0	<LOQ	--
	401	393	98
	401	382	95
	401	399	99
	802	785	98
	1603	1601	100
	3206	3223	101
	6413	6346	99
	6413	6388	100
	6413	6434	100
	6413	6440 ^c	100
January 20, 2012	0	<LOQ	--
	401	386	96
	401	394	98
	401	391	98
	802	792	99
	1603	1631	102
	3206	3158	99
	6413	6382	100
	6413	5778	90
	6413	6394	100

^a Measured concentration in analytical samples collected from spray mixtures.

^b The method limit of quantitation (LOQ) for the analysis of daminozide was set at 25 mg a.s./L, calculated as the product of the concentration of the lowest calibration standard (0.0500 mg a.s./L) and the dilution factor of the matrix blank sample (500).

^c Sample collected from separate spray mixture, therefore measured concentration not included in mean measured calculation.

Conclusion:

A pre-emergent, soil surface application of daminozide formulation using concentrations of up to 7500 ppm applied at nominal rates of 2037 litres of solution per hectare resulted in no adverse effects on the emergence, survival, height, dry weight, and plant condition of the ten species tested. Therefore, the NOER was the highest test level, a test substance concentration of 7500 ppm, and the LOER was greater than 7500 ppm. Since a treatment-group reduction of 25% or greater relative to control means was not observed, the ER₂₅ and ER₅₀ for all parameters were greater than 7500 ppm.

RMS comments and conclusion:

The reported study is GLP compliant and was conducted according to OECD 208 (2006) and OPPTS 850.4225 (1996) guidelines. The test results are in compliance with the guidelines' validity criteria: the seedling emergence was more than 70% (actual value was 95-100%); there was more than 90% survival in the control seedlings (actual value was 95-100%); the environmental conditions were identical for all the tested species; the control plants did not exhibit any phytotoxic effects. The study is acceptable for regulatory use.

The ER₅₀ for all parameters were greater than than 7500 ppm formulation, equivalent to 6413 ppm a.s. which is equivalent to 13 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 ¹	All species were equivalent / all parameters	>7.5 g a.s./ha *	Bramby-Gunary (2015a) ACE-14-159
Dazide Enhance (FAL 2400)	Vegetative vigour ¹	Tomato / dry weight	>4.5 kg a.s./ha*	Bramby-Gunary (2015b) ACE-15-075
Alar 85 WSG	Vegetative vigour	Soybean / height	>7500 ppm product; equivalent to >6413 ppm a.s;	Sindermann et al (2012b) 616-108

Test Substance	Study type	Most sensitive species / parameter	ER ₅₀	Reference
			equivalent to 13 kg a.s./ha *	
Alar 85 WSG	Seedling 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) 616-107

* The highest concentration tested.

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

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 plans 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 10.6.2/01	Sindermann, A.B., Poch, J.R., Krueger, H.O., Martin, K.H.	2012a	Daminozide formulation: A toxicity test to determine the effects (Tier II) on seedling emergence of ten species of plants Wildlife International Ltd. Report No. 616-107 GLP Unpublished	N	Y	New study for AIR 3 dossier	Arysta LifeScience Great Britain Limited .
CP 10.6.2/02	Sindermann, A.B., Poch, J.R., Krueger, H.O., Martin, K.H.	2012b	Daminozide formulation: A toxicity test to determine the effects (Tier II) on vegetative vigour of ten species of plants Wildlife International Ltd. Report No. 616-108 GLP Unpublished	N	Y	New study for AIR 3 dossier	Arysta LifeScience Great Britain Limited .

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 10.1.1.1/01	██████	1966a	Acute dietary toxicity: Bobwhite quail Alar-85 and D-198 ████████████████████ Report No. A.7.4.2.1 Non-GLP Unpublished	Y	N	Not applicable	Arysta LifeScience Great Britain Limited .
CP 10.2.1.1/01	██████	1966b	Acute toxicity in aqueous exposure to rainbow trout: Alar-85 and F461-75W ████████████████████ Report No. A.7.4.1.1 Non-GLP Unpublished	Y	N	Not applicable	Arysta LifeScience Great Britain Limited .

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 10.2.1.1/02	██████	1966c	Acute toxicity in aqueous exposure to bluegill sunfish ████████████████████. Report No. A.7.4.1.2 Non-GLP Unpublished	Y	N	Not applicable	Arysta LifeScience Great Britain Limited .
CP 10.3.1.1.1/ 01	Cole, J.	1985	The acute oral and contact toxicity to honey bees of Alar 85 Huntingdon research Centre. Report No. A.7.4.2.7 GLP Unpublished	N	N	Not applicable	Arysta LifeScience Great Britain Limited .
CP 10.3.1.1.2/ 01	Cole, J.	1985	The acute oral and contact toxicity to honey bees of Alar 85 Huntingdon research Centre. Report No. A.7.4.2.7 GLP Unpublished	N	N	Not applicable	Arysta LifeScience Great Britain Limited .
CP 10.3.2.1/01	Baxter, I.	1999a	A laboratory test to determine the effect of ALAR 85 SP (an 850 g/kg water soluble powder formulation of daminozide) on the parasitic wasp <i>Aphidius rhopalosiphi</i> Mambo-Tox Ltd. Report No. UNI-99-9 GLP Unpublished	N	N	Not applicable	Arysta LifeScience Great Britain Limited .
CP 10.3.2.1/02	Vinall, S.	1999	A laboratory test to determine the effect of ALAR 85 SP (an 850 g/kg water-soluble powder formulation of daminozide) on the predatory mite, <i>Typhlodromus pyri</i> Mambo-Tox Ltd. Report No. UNI-99-8 GLP Unpublished	N	N	Not applicable	Arysta LifeScience Great Britain Limited .
CP 10.3.2.1/03	Halsall, N.	2000	A laboratory test to determine the effect of ALAR 85 SP (an 850 g/kg water-soluble powder formulation of daminozide) on the whitefly parasitoid <i>Encarsia formosa</i> Gahan (Hymenoptera, Aphelinidae) Mambo-Tox Ltd. Report No. UNI-00-2 GLP Unpublished	N	N	Not applicable	Arysta LifeScience Great Britain Limited .
CP 10.3.2.1/04	Vinall, S.	2000	A laboratory test to determine the effect of ALAR 85 SP (an 850 g/kg water-soluble powder formulation of daminozide) on the predatory bug <i>Orius laevigatus</i> Mambo-Tox Ltd. Report No. UNI-00-3 GLP	N	N	Not applicable	Arysta LifeScience Great Britain Limited .

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
			Unpublished				
CP 10.3.2.1/05	Baxter, I.	1999b	A laboratory test to determine the effect of ALAR 85 SP (an 850 g/kg water-soluble powder formulation of daminozide) on the ground beetle, <i>Poecilus cupreus</i> Mambo-Tox Ltd. Report No. UNI-99-10 GLP Unpublished	N	N	Not applicable	Arysta LifeScience Great Britain Limited .
CP 10.3.2.1/06	Barton, R.	1999	A laboratory test to determine the effect of ALAR 85 SP (an 850 g/kg water-soluble powder formulation of daminozide) on the green lacewing <i>Chrysoperla carnea</i> Mambo-Tox Ltd. Report No. UNI-99-11 GLP Unpublished	N	N	Not applicable	Arysta LifeScience Great Britain Limited .
CP 10.3.2.2/01	Tazura, S.	2001a	An extended laboratory test to determine the effect of ALAR 85 SP (an 850 g/kg water-soluble powder formulation of daminozide) on the predatory mite <i>Typhlodromus pyri</i> (Acari, Phytoseiidae) Mambo-Tox Ltd. Report No. UNI-01-1 GLP Unpublished	N	N	Not applicable	Arysta LifeScience Great Britain Limited .
CP 10.5/01	Maas, G.	1987 1989	Effects on soil non-target organisms Report No. A.8.1.18 Non-GLP Unpublished	N	N	Not applicable	Arysta LifeScience Great Britain Limited .