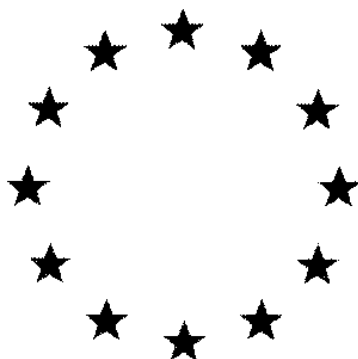


# **European Commission**



**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.8 (AS)**

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

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## B.8 Environmental fate and behaviour

The active substance Daminozide (ISO); 4-(2,2-dimethylhydrazino)-4-oxobutanoic acid; N-dimethylaminosuccinamic acid ('hereafter referred to as 'daminozide') is used as a plant growth regulator. The representative uses are for a maximum of 5 applications to ornamental crops grown either indoors or in the field. The maximum individual application rates are 7.65 kg a.s./ha made indoors or 4.25 kg a.s./ha made in the field.

The structure of daminozide is shown in Figure B.8-1.

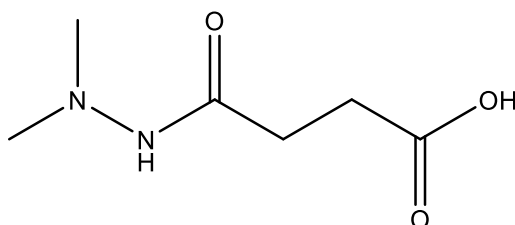


Figure B.8-1: The structure of daminozide

**B.8.1 Fate and behaviour in soil****B.8.1.1 Route and rate of degradation in soil****B.8.1.1.1 Route of degradation in soil****B.8.1.1.1.1 Aerobic degradation**

<b>Reference:</b>	<b>Dannals, L.; Puhl, R.; Kucharczyk, N. (1972)</b> Environmental Fate Studies on Alar: Second Status Report on PR 70-15
Report No.:	A.8.1.3
Document No:	-
Guideline:	-
GLP:	no
<b>Previous evaluation:</b>	In DAR (1999)

**Material and methods:**

Test material: Alar-<sup>14</sup>C (methyl tag)

Specific radioactivity: 1.3 mCi mmol<sup>-1</sup>

Radiochemical purity: >95%

Sample/Batch ID: -

The potassium salt was prepared from Alar-<sup>14</sup>C in water by addition of K<sub>2</sub>CO<sub>3</sub>

**Table B.8.1.1.1.1-1: Analyses of soils used**

Location	Classification based on analysis	Sand <sup>a</sup>	Silt <sup>a</sup>	Clay <sup>a</sup>	Organic matter <sup>b</sup>	pH	CEC me/100g <sup>c</sup>
Bethany, Conn.	sandy loam	52.6	33.0	14.4	5.8	5.0	9.0
Mississippi	silt loam	11.6	68.0	20.4	4.2	6.4	13.8
Illinois	clay loam	39.6	32.0	28.4	12.8	7.0	25.7
North Carolina (peanut field)	sandy loam	59.6	32.0	8.4	2.2	5.8	5.3

<sup>a</sup> Air dry basis, Bouyoucos hydrometer method

<sup>b</sup> Loss-on-ignition, water-free basis

<sup>c</sup> Cation exchange capacity, air dry basis, copper acetate method

In non-sterile soil experiments, the Alar-<sup>14</sup>C or its K-salt and water, if any, were injected onto the soil surface before assembly of the apparatus. In the sterile soil experiment the apparatus was assembled with soil in the flask but without the NaOH solution and with septums removed. It was placed in an oven with hypodermic syringes and a 50 ml graduate. The oven was heated to 121 – 127 °C for 30 minutes and allowed to cool to 50°C. The oven was opened, NaOH solution charged and septums inserted as rapidly as possible. The Alar-<sup>14</sup>C in sterile water was injected onto the soil surface through the septum. The NaOH solution was sampled with a hypodermic syringe and sterilized needle through the septum after various periods of exposure. Determination of <sup>14</sup>C radioactivity was

done by liquid scintillation counting (LSC) in a Beckmann LS-250, using 0.1 ml of the NaOH solution in 15 ml of xylene-methanol-phenethylamine-PPO counting solution.

Determination of Alar in the extractable soil residue: The propanol-water-ammonia extract of the soil contained 5% of the originally applied  $^{14}\text{C}$  activity. The residue was analysed by the procedure of the colorimetric Alar test. The distillate was counted by LSC and was found to contain 80% of the  $^{14}\text{C}$  activity extracted. The color test confirmed the activity was present in the distillate as dimethyl hydrazine.

It may be concluded that for both Alar and its potassium salt on sandy loam, 85% of the applied radioactivity has disappeared in 14 days, and only about 12% of the applied radioactivity is left as bound (unextracted) residue after this time (see table below). The rate of dissipation for both, based on degradation to  $^{14}\text{CO}_2$ , is approximately the same.

Results presented in table B.8.1.1.1.1-3 show that both Alar and its K-salt on sandy loam are converted rapidly to  $^{14}\text{CO}_2$  since 60% of the applied radioactivity is found in the NaOH solution after 4 days and about 80% after 13 or 14 days.

Degradation on sterilised soil (table B.8.1.1.1.1-4): Microbial degradation is involved, as shown by an experiment using the closed system with Alar- $^{14}\text{C}$  on heat sterilised sandy loam. After 7 days exposure, only 0.2% of the applied radioactivity was found in the NaOH solution, as compared with 67% in an equivalent experiment with unsterilized soil.

**Table B.8.1.1.1.1-2: Residues of  $^{14}\text{C}$  radioactivity in sandy loam two weeks after treatment with Alar- $^{14}\text{C}$  and its K-salt**

	Average % of applied $^{14}\text{C}$ radioactivity				
	Sandy loam No.	Exposure time (days)	Extractable with propanol-water- $\text{NH}_3$	Unextracted (determined by dry combustion)	Total left on soil
Alar	B-1	14	5.0	11.8	16.8
K-Salt	B-1	13	2.7	12.3	15.0
K-Salt	N-2	14	2.3	12.5	14.8

**Table B.8.1.1.1.1-3: Rate of degradation of Alar- $^{14}\text{C}$  and its K-Salt to  $^{14}\text{CO}_2$  on sandy loam**

Time in days	% of applied $^{14}\text{C}$ activity trapped as $^{14}\text{CO}_2$	
	Alar	K-salt
1	23	40
2	40	63
3	52	-
4	60	-
7	67	73
9	73	75
13	-	84
14	75	-

**Table B.8.1.1.1-4: Alar-<sup>14</sup>C degradation on sterilised vs. non-sterile sandy loam**

	Exposure (days)	Extractable from soil with propanol-water-NH <sub>3</sub>	Average % of applied <sup>14</sup> C radioactivity		
			In extracted soil (by dry combustion)	In NaOH solution	Total
Sterilized	10	85.1*	7.8	0.2	93.1
Non-sterile	14	5.0**	11.8	75.4	92.2

\* 92% Alar by thin-layer chromatography

\*\* more than 80% of this amount was determined to be Alar by the colorimetric test.

### RMS comments and conclusion

The soil dissipation and mineralisation study of <sup>14</sup>C-daminozide was not conducted according to any guideline. A review of this study indicates that it does not meet the current OECD 307 (2002) guideline. Results in sandy loam soil are reported only. Moreover, samples were analysed after 13 or 14 days, no sampling at t=0, no details on metabolites or total balance are reported. No information on the storage conditions or the history of the soil material before its use in the degradation experiment, soil moisture content is unknown. Study is not considered acceptable. Results are not useful and are not used for risk evaluation, except for the information that under sterile conditions the transformation is inhibited.

<b>Reference:</b>	<b>Dannals, L.; Puhl, R.; Kucharczyk, N. (1974)</b> Dissipation and Degradation of Alar® in Soils Under Greenhouse Conditions
Report No.:	Not provided
Document No:	-
Guideline:	-
GLP:	No
<b>Previous evaluation:</b>	In DAR (1999)

### Material and methods:

Test material: Alar-<sup>14</sup>C (methyl tag)

Specific radioactivity: 1.3 mCi mmol<sup>-1</sup>

Radiochemical purity: 98%

Sample/Batch ID: -

Alar-<sup>14</sup>C (carbonyl tagged) was prepared similarly in approximately the same yield and purity.

### Description

Soil dissipation and mineralisation of <sup>14</sup>C-daminozide was studied in four soils in the greenhouse. Descriptions and analyses of four soils used in our experiments are given in table below.

**Table B.8.1.1.1-5: Chemical and physical characteristics of soils used**

Location	Classification based on analysis	Sand <sup>a</sup>	Silt <sup>a</sup>	Clay <sup>a</sup>	Organic matter <sup>b</sup>	pH	CEC me/100g <sup>c</sup>
Connecticut	sandy loam	52.6	33.0	14.4	5.8	5.0	9.0
Mississippi	silt loam	11.6	68.0	20.4	4.2	6.4	13.8

Illinois	clay loam	39.6	32.0	28.4	12.8	7.0	25.7
North Carolina	sandy loam	59.6	32.0	8.4	2.2	5.8	5.3

<sup>a</sup> Air dry basis, Bouyoucos hydrometer method

<sup>b</sup> Loss-on-ignition, water-free basis

<sup>c</sup> Cation exchange capacity, air dry basis, copper acetate method

Dissipation: The soil samples (70-90 g) were placed in plastics pots in a greenhouse and an aqueous solution of N-methyl-<sup>14</sup>C-daminozide (specific activity 0.2 mCi/mM) solution was applied evenly to the surface with a syringe at a rate equivalent to 0.85 lb active ingredient/acre. The soils were kept at 20 to 30°C and watered twice a week. Duplicate soil samples were taken after 4, 7, 14, 28, 38 and 120 days, stirred for 2 hours and extracted with a 7:3 mixture of 1-propanol/water, containing one volume percent of aqueous ammonia.

With freshly treated soils this extraction gave quantitative recoveries in two soils, of 87% in clay loam and 67% in the silty loam. CO<sub>2</sub> was not trapped. Soils were combusted. A dissipation study with carbonyl-<sup>14</sup>C labelled daminozide was also carried out in the sandy loam soil.

The mineralisation of N-methyl-<sup>14</sup>C labelled daminozide was also studied in the laboratory in sterile and non-sterile sandy loam soils (100 g sample, 16 mg/kg, applied as free acid and as potassium salt). <sup>14</sup>CO<sub>2</sub> was trapped in NaOH. After completion of the experiment, soil was extracted and combusted. The soil was sterilized by heating to 121-127 °C for 30 min. The bound residue in the non-sterile soil was fractionated. Detec. method: TLC and LSC (81-89% counting efficiency).

## Results

Dissipation: The total extractable <sup>14</sup>C was 6-23% after 4 days and decreased to less than 9% in 7 days. <sup>14</sup>C-disappearance curves were similar for the two labels in the sandy loam soil. Since the rate of disappearance from the soil is similar in both soils it is evident that complete degradation of daminozide occurs rapidly. After 4 weeks the total <sup>14</sup>C residue in all four soils was less than 27% of the amount applied. Bound residues (i.e. non-extractable r.a.) amounted to a maximum of 27% after 4 days in the clay loam, dropping to 25% after 28 days (not further measurements), see table below.

**Table B.8.1.1.1-6: Residues of <sup>14</sup>C in soils after ALAR-<sup>14</sup>C treatment**

Fraction	Time (days)	Average % of applied <sup>14</sup> C				
		Carbonyl tag	N-Methyl tag			
		Connecticut sandy loam	Connecticut sandy loam	N. Carolina sandy loam	Illinois clay loam	Mississippi silt loam
Extractable	4	-	8.6	12.9	22.9	6.1
	7	7.0	4.2	5.6	8.8	5.5
	14	5.3	3.2	5.1	3.9	3.1
	28	-	-	4.4	2.0	2.5
	38	2.8	2.4	-	-	-
	120	-	3.6	-	-	-
Unextractable	4	-	21.7	8.2	27.1	15.3
	7	16.8	14.3	10.0	26.0	12.5
	14	7.0	9.5	8.4	25.9	15.9
	28	-	-	9.3	24.8	12.3



	38	5.8	9.1	-	-	-
	120	-	5.8	-	-	-
Total	4	-	30.3	21.1	50.0	21.4
	7	23.8	18.5	15.6	34.8	18.0
	14	12.3	12.7	13.5	29.8	19.0
	28	-	-	13.7	26.8	14.8
	38	8.6	11.5	-	-	-
	120	-	9.4	-	-	-

Mineralisation: After 14 days 5% of the initially applied  $^{14}\text{C}$  (N-methyl tag) was extractable from the non-sterile sandy loam (1) soil (of which 80% daminozide), 75.4% was trapped in NaOH, and 11.8% was soil bound residue (determined by combustion). The volatiles were identified as  $\text{CO}_2$ .

After 10 days 85.1% was extractable in the sterile soil (92% daminozide), 0.2% was trapped in NaOH, and 7.8% was found as bound residue. Apparently microbial activity causes transformation. The soil after extraction with propanol mixture was extracted with NaOH, which yielded 10% of the applied r.a.. This r.a. was divided over humic and fulvic fraction in the ratio 1:2, see table below.

**Table B.8.1.1.1-7: ALAR- $^{14}\text{C}$  (N-methyl tag) degradation on sterilized vs. non-sterile sandy loam**

	Exposure (days)	Average % of applied $^{14}\text{C}$ radioactivity			
		Extractable from soil with propanol-water- $\text{NH}_3$	In extracted soil (by dry combustion)	In NaOH trapping solution	Total
Sterilized	10	85.1 <sup>a</sup>	7.8	0.2	93.1
Non-sterile	14	5.0 <sup>b</sup>	11.8	75.4	92.2

<sup>a</sup> 92% ALAR by thin-layer chromatography

<sup>b</sup> Eighty percent of this amount was determined to be ALAR by the colorimetric test (Lane 1967)

### RMS comments and conclusion

The soil dissipation and mineralisation study of  $^{14}\text{C}$ -daminozide was not conducted according to any guideline. A review of this study indicates that it does not meet the current OECD 307 (2002) guideline, main deviations include: No exact temperature is reported (only between 20-30°C); moisture content is unknown. No sampling at  $t = 0$ . No distribution of extractable radioactivity was analysed, no information on metabolites formed, total mass balance is unknown. Concentration used in the four soils (dissipation) cannot be calculated. No information on the storage conditions or the history of the soil material before its use in the degradation experiment. Study is not considered acceptable. Results are not useful and are not used for risk evaluation, except for the information that under sterile conditions the transformation is inhibited.

<b>Reference:</b>	<b>Dzialo, D.; Harned, W. (1986)</b> Daminozide aerobic and anaerobic soil metabolism studies
Report No.:	A.8.1.5
Document No:	-
Guideline:	EPA registration standard (June 29, 1984)
GLP:	No
<b>Previous evaluation:</b>	In DAR (1999)

**Material and methods:**

Test material: methyl-<sup>14</sup>C-daminozide

Specific radioactivity: 5.682 mCi mmol<sup>-1</sup>

Radiochemical purity: 96.1%

Sample/Batch ID: #83-78-97

**Table B.8.1.1.1-8: Chemical and physical characteristics of soils used**

Soil texture	Sandy loam
% sand	67.6
% Silt	26.0
% Clay	6.4
pH	5.6
% organic matter	7.34
% moisture retention at 1/3 Bar	26.88
Cation Exchange Capacity (CEC) (mmol/kg)	435

### Description

A laboratory soil transformation study was performed with methyl-<sup>14</sup>C-daminozide (96.1% pure) under aerobic and anaerobic conditions at 25°C. Soil samples (25 g dw, in duplicate) were incubated in the dark at fortification levels of 6.72 mg/kg (aerobic) and 8.64 mg/kg (anaerobic). Volatile CO<sub>2</sub> was trapped in ascarite layers. Sampling at t = 0, 3, 6, 7, 24, and 48 hours.

After 16 hours anaerobic conditions were established by flooding with distilled water. Zero time samples were taken immediately after establishing anaerobic conditions. Sampling at t = 0, 15, 30, and 60 days.

Extraction with propanol/water/ammonia, remaining soil was dried and combusted. Analysis with LSC, HPLC-r.a. detection, and GC with FID (analytical recoveries not reported).

### Results

Total recovery r.a. dropped from 103% at t = 0 to 62.7% at t = 48 hours. DT50 aerobic incubation 17.3 hours, r<sup>2</sup> 0.986. Three minor metabolites were observed, all <1%. Bound residues and CO<sub>2</sub> amounted to 25 and 20% after 48 hours (end).

Anaerobic: total recovery r.a. dropped from 82.4% at t = 0 to 42.2% at t = 60 days. DT50 anaerobic incubation 7.5 days, based on two timepoints. Bound residues and CO<sub>2</sub> amounted to maxima of 25 and 20% after 15-30 days, and to 22 and 17% after 60 days (end). Formaldehyde amounted to 0.1 mg/kg after 30 days (equal to 5% of r.a. recovery after flooding), 0.07 mg/kg after 60 days. Dimethylamine was found as a minor metabolite directly after flooding. No UDMH (1,1-dimethylhydrazine) or NDMA (1,1-dimethylnitrosamine) were observed in both systems.

Part of the losses in the mass balance are due to the formation of methane and ethane and to the loss of bicarbonate (as CO<sub>2</sub>) in the analysis procedure (pH adjustment to 3.5).

**Table B.8.1.1.1.1-9: Summary of total  $^{14}\text{C}$  distribution in sandy loam under aerobic conditions**

Time (hours)	% of applied $^{14}\text{C}$				
	Extractable	Bound	$\text{CO}_2$	Volatiles	Total $^{14}\text{C}$ recovered <sup>a</sup>
0	92.6	9.9	N.A. <sup>b</sup>	N.A. <sup>b</sup>	102.5
3	79.8	11.8	0.7	0.03	92.3
6	70.5	17.9	5.6	0.04	94.0
7	66.0	16.6	6.1	0.06	88.8
24	47.5	21.5	18.5	0.15	87.7
48	16.1	25.0	20.0	1.6	62.7

<sup>a</sup> Total = extractable + bound +  $\text{CO}_2$  + volatile<sup>b</sup> N.A. = Not analysed

\* For conversion to ppm: treatment rate =  $168.12 \mu\text{g } ^{14}\text{C-daminozide}/25 \text{ g soil}$   
 $= 6.72 \mu\text{g } ^{14}\text{C-daminozide}/ \text{g soil} = 6.72 \text{ ppm}$

ppm = [(% of applied  $^{14}\text{C}$ ) (6.72 ppm)]/100

**Table B.8.1.1.1.1-10: Aerobic soil metabolism: characterisation of extractable residues (ppm)<sup>a</sup>**

Time (hours)	Total extractable	Extractable as daminozide	HPLC Retention time – 36.5 min.
0	6.22	5.95	N.D. <sup>b</sup>
3	5.36	5.16	N.D. <sup>b</sup>
6	4.74	4.56	0.03
7	4.44	3.63	0.05
24	3.19	2.35	0.01
48	1.08	0.82	0.01

<sup>a</sup> Three minor metabolites were inconsistently detected at some sampling periods, none of which exceeded 0.06 ppm. Identification was not possible because of low levels of radioactivity.

<sup>b</sup> N.D. = Not detected**Table B.8.1.1.1.1-11: Summary of total  $^{14}\text{C}$  distribution in sandy loam under anaerobic conditions (% AR\*)**

Time (days)	Aqueous <sup>b</sup> filtrate	Extractable <sup>b</sup> residue	Bound	$^{14}\text{CO}_2^c$	$^{14}\text{CO}_2^d$	Volatile <sup>e</sup>	Total recovery
0 <sup>a</sup>	19.4	25.4	21.8	13.8	0.7	1.3	82.4
15	2.1	2.6	24.9	13.8	5.9	1.3	50.6
30	0.5	2.0	25.3	13.8	3.5	1.3	46.4
60	0.7	1.5	21.9	13.8	3.0	1.3	42.2

<sup>a</sup> Time after establishing anaerobic conditions<sup>b</sup> Adjusted for loss of  $^{14}\text{CO}_2$  which occurs with change of pH<sup>c</sup> Average of  $^{14}\text{CO}_2$  traps analysed at zero time<sup>d</sup> Sum of  $^{14}\text{CO}_2$  released from aqueous filtrate and extractable residues<sup>e</sup> Average of volatile traps analysed at zero time

\* For conversion to ppm: treatment rate =  $215.91 \mu\text{g } ^{14}\text{C-daminozide}/25 \text{ g soil}$   
 $= 8.64 \mu\text{g } ^{14}\text{C-daminozide}/ \text{g soil} = 8.64 \text{ ppm}$

ppm = [(% of applied  $^{14}\text{C}$ ) (8.64 ppm)]/100

**Table B.8.1.1.1-12: Anaerobic soil metabolism: Characterisation of aqueous residues and extractable residues (ppm)**

Aqueous filtrate time (days)	Total <sup>14</sup> C	As daminozide	As formaldehyde	As dimethylamine
0 <sup>c</sup>	1.68	1.51	0.05	0.05
15	0.18	0.07	0.02	N.D. <sup>b</sup>
30	0.04	N.A. <sup>a</sup>	N.A. <sup>a</sup>	N.A. <sup>a</sup>
60	0.06	N.A. <sup>a</sup>	N.A. <sup>a</sup>	N.A. <sup>a</sup>
Extractable residues time (days)	Total <sup>14</sup> C	As daminozide	As formaldehyde	As dimethylamine
0 <sup>c</sup>	2.19	1.65	0.07	0.04
15	0.22	0.01	0.09	N.D. <sup>b</sup>
30	0.17	N.D. <sup>b</sup>	0.10	N.D. <sup>b</sup>
60	0.13	N.D. <sup>b</sup>	0.07	N.D. <sup>b</sup>

<sup>a</sup> N.A. = Not analysed due to low level of total <sup>14</sup>C

<sup>b</sup> N.D. = Not detected

<sup>c</sup> Three minor metabolites at 29.0, 34.5 and 36.5 minutes were detected at zero time, none of which exceeded 0.02 ppm.

#### **RMS comments and conclusion:**

Study was not performed according to current guideline and not GLP. A review of this study indicates that it does not meet the current OECD guideline, main deviations include:

- Total recovery was in the recommended range up to 6 hours only under aerobic conditions, in anaerobic experiment total mass balance was below 90 % at all sampling points and decreased to 42.2 % AR after 60 hours.
- There is no information on the storage conditions or the history of the soil material before its use in the degradation experiment. Organic matter content of the soil was very high.
- Recoveries analytical methods not reported.

Study is not considered acceptable and no endpoint will be used for the risk assessment.

<b>Reference:</b>	<b>Goodyear, A. (1995)</b> ( <sup>14</sup> C)-Daminozide Soil metabolism and degradation
Report No.:	1137/2-1015
Document No:	-
Guideline:	Dutch guidelines for the submission of applications for registration of pesticides, Part G (June 1991), EPA Pesticides Assessment Guidelines, Subdivision N Paragraph 162-1 (October 1982)
GLP:	Yes
<b>Previous evaluation:</b>	In DAR (1999)

#### Material and methods:

Test material: <sup>14</sup>C-daminozide

Specific radioactivity: 24.6 mCi/mmol

Radiochemical purity: > 98%

Sample/Batch ID: CSL-94-538-06-19

#### Description

The objectives of the study were to determine the rate and route of degradation of (<sup>14</sup>C)-daminozide (N-dimethylaminosuccinamic acid) in two soils (Speyer 2.2 and Colorado clay loam) and the rate of degradation of (<sup>14</sup>C)-daminozide in a further two soils (Speyer 2.1 and California US silt loam). Soil characterisation is presented in the table B.8.1.1.1.1-13. All soils were homogenised by passing through a 2 mm sieve prior to use.

Sampling was performed up to and including 64 days for each soil type, with additional samples of the Speyer 2.2 and Colorado clay loam removed at 77 days to further investigate the radioactive material balance. All studies were maintained under aerobic conditions in the dark at 20 ± 1°C.

**Table B.8.1.1.1-13: Soil characterisation**

Name	Speyer 2.1	Speyer 2.2	Colorado	California
Origin	Speyer, Germany	Speyer, Germany	USA	USA
Classification (BBA/USDA)	sand	loamy sand	clay loam	silt loam
pH (H <sub>2</sub> O)	5.9	6.0	8.0	5.9
pH (KCl)	5.5	5.6	7.2	4.1
Organic matter (%)	0.7	2.9	1.2	1.6
Organic carbon (%)	0.4	1.7	0.7	0.9
CEC (meq/100g soil)	4.2	11.5	17.3	19.0
Moisture holding capacity at 0.33 bar	4.7	16.2	23.1	26.4
Pre-application biomass (µgC/g soil)	83	473	151	75
Post-application biomass (µgC/g soil)	76	487	239	78

Soil samples (50 g on dry weight basis, in duplicate) were incubated in the dark at fortification levels of 1.5 mg/kg (samples were mixed). Soil sampling at t = 0, 8, 24, 32h and 2, 4, 8, 16, 32, and 64 days (two treatments were analysed after 77 days as well).

Soil moisture was adjusted to the 0.33 Bar water holding capacity, prior to incubation, with the exception of the Colorado clay loam soil which was maintained at 75% of the 0.33 Bar moisture holding capacity. Samples of the Speyer 2.2 and Colorado clay loam soils were maintained in a common chamber and throughout the experiment moistened carbon dioxide free air was drawn over the soils, before being passed through a series of trapping reagents designed to collect volatile degradation products.

Extraction with methanol (0h) or methanol/ammonia (other timepoints), NaOH (50°C) and methanol prior to combustion. Analysis by LSC, HPLC-UV and TLC. Bound residue of 24h and 16d samples in all four soils was fractionated with HCl and NaOH, followed by combustion. Analysis with LSC. Moist carbon free air was drawn over the loamy sand and clay loam samples into four traps (paraffin in xylene for non-polar volatiles and NaOH for CO<sub>2</sub>). Analysis with LSC.

Recoveries of analytical methods were not reported.

## Results

No mass balance for sand and silty loam is available (no CO<sub>2</sub> nor bound residue measured). Loamy sand and clay loam: total recovery r.a. dropped from 95% at t= 0 to 63-70% at t = 64 days. In sealed flasks that were only sampled after 77 days the total recovery was 75-77% after 77 days.

CO<sub>2</sub> amounted to 43-45% after 64 days (end), and to 55-57% in the sealed vessels after 77 days in loamy sand and clay loam. The maximum bound residues determined for the Speyer 2.2 was 13.4% after 32 hours and 9.2 after 64 days (end). Extraction with methanol/ammonia only released 6-44% of r.a. in 0-8h samples, and declining amounts

up to <1% after 64 days. Extraction with NaOH released r.a. associated with humic and fulvic acid, leading to efficiencies of 50-74% shortly after incubation to 7-12% after 64 days. Due to the choice of solvents the amount of daminozide after 0h (8-10%) is lower than after 8 hours (15-37%). Metabolites were <3% at all timepoints. Of bound residue in sand and silt loam 18% of r.a. applied was in NaOH extract, of which 75-80% was in fulvic acid, rest in humic acid (humins not determined). Of bound residue in loamy sand (15-18%) and clay loam (8-33%) about half of the r.a. was in humin fraction, the other half was distributed over fulvic and humic acid as in other soils. DT50 were calculated with  $Y=abx$  to 0.1-1.1 days (based on methanol/ammonia extracts).

**Table B.8.1.1.1-14: Percent recovery of applied radioactivity from Speyer 2.1 (sand) soil following  $^{14}\text{C}$ -daminozide treatment**

Time	Mass balance (MeOH/NH <sub>3</sub> + NaOH extracts+MeOH wash)	HPLC characterisation of methanol:ammonia extracted radioactivity			
		Final extract	daminozide	unknowns	Unresolved background
0 hour	94.5	11.0	9.6	1.3	0.1
8 hour	69.6	39.8	36.7	2.7	0.4
24 hour	38.4	13.4	10.9	2.4	0.1
32 hour	27.7	-	-	-	-
2 day	23.6	-	-	-	-
4 day	21.0	-	-	-	-
8 day	18.1	-	-	-	-
16 day	17.6	-	-	-	-
30 day	15.5	-	-	-	-
64 day	14.5	-	-	-	-

**Table B.8.1.1.1-15: Percent recovery of applied radioactivity from Speyer 2.2 (loamy sand) soil following  $^{14}\text{C}$ -daminozide treatment**

Time	Total extracted (MeOH/NH <sub>3</sub> + NaOH extracts+MeOH wash)	Carbon dioxide	Bound residue	Mass balance	HPLC characterisation of methanol:ammonia extracted radioactivity			
					Final extract	daminozide	unknowns	Unresolved background
0 hour	87.1	N.A.	9.8	96.9	9.5	8.4	1.0	<0.1
8 hour	54.7	17.	9.6	81.2	16.4	14.9	1.3	0.2
24 hour	28.4	34.1	12.2	74.7	-	-	-	-
32 hour	23.9	36.6	13.4	73.9	-	-	-	-
2 day	22.1	38.1	12.9	73.1	-	-	-	-
4 day	21.1	39.8	11.9	72.8	-	-	-	-
8 day	19.7	41.2	11.0	71.9	-	-	-	-
16 day	18.5	42.4	11.2	72.1	-	-	-	-
30 day	18.0	43.4	10.4	71.7	-	-	-	-
64 day	16.5	44.7	9.2	70.4	-	-	-	-



**Table B.8.1.1.1-16: Percent recovery of applied radioactivity from California (silt loam) soil following  $^{14}\text{C}$ -daminozide treatment**

Time	Mass balance (MeOH/NH <sub>3</sub> + NaOH extracts+MeOH wash)	HPLC characterisation of methanol:ammonia extracted radioactivity			
		Final extract	daminozide	unknowns	Unresolved background
0 hour	75.0	6.2	4.1	2.1	<0.1
8 hour	27.1	-	-	-	-
24 hour	17.8	-	-	-	-
32 hour	15.5	-	-	-	-
2 day	15.8	-	-	-	-
4 day	14.2	-	-	-	-
8 day	12.2	-	-	-	-
16 day	10.9	-	-	-	-
30 day	10.5	-	-	-	-
64 day	9.7	-	-	-	-

**Table B.8.1.1.1-17: HPLC characterisation of methanol:ammonia extracted radioactivity from Colorado clay loam soil following ( $^{14}\text{C}$ )-daminozide treatment**

Sampling	final extract	daminozide	unknowns	unresolved background
8 hour	10.8	9.0	1.7	<0.1
24 hour	7.1	5.6	1.5	0.1

**Table B.8.1.1.1-18: Percent recovery of applied radioactivity after 77 days incubation at 20°C from a Speyer 2.2 and Colorado clay loam soil following ( $^{14}\text{C}$ )-daminozide treatment**

Soil	MeOH/NH <sub>3</sub> extract	CO <sub>2</sub> trap 1	CO <sub>2</sub> trap 2	Bound residue	Mass balance
77 day Speyer 2.2	0.4	48.1	9.0	20.0	77.4
77 day Colorado	0.3	47.3	7.3	20.3	75.2

**Table B.8.1.1.1-19: DT<sub>50</sub> and DT<sub>90</sub> values for daminozide determined from methanol:ammonia (95:5 v/v) extracts**

Soil	DT <sub>50</sub> (days)	DT <sub>90</sub> (days)
Speyer 2.1	0.1	1.1
Speyer 2.2	<0.1	0.3
California silt loam	<0.1	0.1
Colorado clay loam	0.1	0.5

**RMS comments and conclusion**

A review of this study indicates that it does not meet the current OECD guideline, main deviations include:

No information on the history of the soil material before its use in the degradation experiment. Recoveries analytical methods are not reported. HPLC characterisation of methanol-ammonia extracted radioactivity was not performed for almost all sampling points. Mass balance was not obtained for the Speyer 2.1 and California silt loam soil as bound residues and volatile products were not determined. Total mass balance dropped below the recommended range of 90 – 110% after 8 hours. Mass balance for Colorado soil was determined after 77 days and was 75.2% only.

The calculation of DT50 and DT90 is based on the data from the methanol:ammonia extract, DT50 and DT90 values have to be based on daminozide residues only.

Study is considered not acceptable, results from this study will not be used further.

<b>Reference:</b>	<b>Yu, W.; Kobryn, K.W. (1993)</b> Daminozide Aerobic Soil Metabolism
Report No.:	A.8.1.21
Project number:	92123
Guideline:	EPA Pesticide Assessment Guidelines, subdivision N, Section 162-1
GLP:	Yes
<b>Previous evaluation:</b>	In DAR (1999)

**Material and methods:**

Test material:  $^{14}\text{C}$ -methyl label daminozide

Specific radioactivity: 24.7 mCi/mmol

Radiochemical purity: > 98%

Lot # CSL-91-344-39-30

**Description**

Paxton sandy loam soil was used in this study. The soil was sieved through a 2 mm screen. Chemical and physical properties of the test soil are presented in table below. Microbial activity of test soil was also assayed to ensure a viable microbial population was present.

**Table B.8.1.1.1-20: Physical and Chemical Properties of Paxton Soil**

% Sand	58
% Silt	30
% Clay	12
% Organic Matter	3.9
C.E.C. (meg/100g)	8.5
pH	6.5
Soil Classification	Sandy loam
Bulk Density (g/cc)	1.28
% Moisture Content	18.86
Field Capacity, 1/3 Bar %	30.97

Approximately 25 grams (25 g on dry weight basis, in duplicate) of Paxton sandy loam was weighted into replicate 250-ml soil flasks. The soil moisture in each flask was adjusted to 75% field capacity at 1/3 bar by the addition of water to the soil. Soil samples were incubated in the dark at fortification levels of 9.07 mg/kg. Soil sampling at  $t = 0, 2, 4, 6, 16, 24, 48$  and 72 hours, in replicates. The soil flasks were incubated at  $25 \pm 1^\circ\text{C}$  in the dark.

Extraction with methanol/formic acid, remaining soil was dried and combusted. Analysis with LSC, HPLC-r.a. detection (analytical recoveries not reported). Bound residue of 48h and 72h samples were fractionated with acetonitrile/water/acetic acid, HCl, and NaOH, and with protease type XIV, followed by combustion. Analysis with LSC.

Volatiles were trapped in ethylene glycol, NaOH and sulfuric acid, and analysed by LSC. The volatiles were collected in the ethylene glycol trap, while the acidic compounds and  $^{14}\text{CO}_2$  were collected in the sodium hydroxide traps, and basic compounds in the 1N sulphuric acid.

## Results

Total recovery r.a. dropped from 95.8% at  $t = 0$  to 84.2% at  $t = 48$  hours. Three minor metabolites were observed, all  $<1\%$ . Bound residues amounted to 23% after 48h and 20% after 72h (end).  $\text{CO}_2$  amounted to 59% after 72 hours (end). Formaldehyde amounted to 0.36 mg/kg after 16h (equal to 21% of r.a. dosed), 0.02 mg/kg after 72h. No UDMH (1,1-dimethylhydrazine) or NDMA (1,1-dimethylnitrosamine), or dimethylamine (DMA) were observed in soil (det. limit 0.01 mg/kg).

Protease released max. 4% r.a. of bound residue after 48-72h, ca. 4% was extractable with acetic acid, 20% was in fulvic fraction, 14% in humic fraction, 2% in humin fraction.

DT50 of daminozide determined using linear regression analysis was 9.5 hours,  $r^2$  0.954.

Table B.8.1.1.1-21: Summary of Material balance

Time, hours	Extractable residues	Protease digestion <sup>a</sup>	Bound residues	Ethylene glycol	1N NaOH	1N H <sub>2</sub> SO <sub>4</sub>	Total % of dose	Average % of dose	Corrected % of dose
0	87.95	-	1.44	-	-	-	89.39	95.81	100.0
	100.34	-	1.89	-	-	-	102.23		
2	92.11	-	7.16	0.29	0.65	0.10	100.31	100.04	104.4
	92.42	-	6.81	0.28	0.25	0.02	99.77		
4	80.71	3.18	7.42	0.90	1.89	0.02	94.11	93.97	98.1
	81.68	2.41	7.29	0.98	1.46	0.01	93.83		
6	77.77	2.39	6.00	2.30	4.47	0.01	92.95	93.69	97.8
	77.96	2.58	7.87	1.49	4.52	0	94.43		
16	37.31	3.87	16.68	7.96	15.75	0.01	81.59	87.61	91.4
	44.67	4.09	16.48	4.29	24.09	0.01	93.62		
24	23.05	4.58	17.22	4.71	38.71	0.03	88.30	85.41	89.2
	19.84	3.97	19.16	2.11	37.44	0.02	82.53		
48	3.44	3.68	23.53	4.20	50.27	0.04	85.16	84.16	87.8
	6.48	3.65	21.77	4.02	47.22	0.02	83.16		
72	2.67	3.57	20.94	1.21	56.24	0.09	84.72	87.97	91.8
	2.67	3.65	19.93	2.57	62.39	0.03	91.22		

<sup>a</sup> Residues solubilized from bound residues in protease fraction

Table B.8.1.1.1-22: Summary of extractable residues

Sampling interval, hours	Daminozide ppm	Formaldehyde ppm
0	8.97	<0.01
2	7.57	0.32
4	7.12	0.34
6	6.30	0.29
16	2.47	0.36
24	1.66	0.01
48	0.72	0.01
72	0.03	0.02

#### RMS comments and conclusion:

A review of this study indicates that it partly meets the current OECD guideline, main deviations include:

No information on the storage conditions or the history of the soil material before its use in the degradation experiment. Recoveries analytical methods not reported. A temperature was 25°C instead of 20°C. Organic matter

content of the soil was very high. Total recovery dropped below the recommended limit of 90 % in all samples after 16 hours. Moreover, there was a problem with identification of a metabolite (see notifier's position below). Due to several deviations the results of this study will not be used further. Study is superseded by the new modern study performed according to OECD test guideline 307 (Möndel, 2015).

#### Notifier's statement to detection of formaldehyde:

*Formaldehyde was reported to have been identified as part of the first review. However, re-examination of the study report of Yu and Kobryn, 1993, in which the presence of formaldehyde was reported, demonstrates that the previous study only utilised HPLC analysis with radio- and UV detection. No confirmatory analytical method was reported and the only reference standard investigated was that for formaldehyde. Therefore, though the formaldehyde standard eluted with a comparable retention time to the metabolite peak in the original study, because the peak was un-retained the degradation product observed in that study could be any polar compound which would also be likely to be un-retained, including methanol. Therefore, the method of analysis in the study of Yu and Kobryn, 1993, does not allow a robust identification of the metabolite observed.*

RMS agrees with the statement provided by the Notifier that the analytical method used in the study by Yu and Kobryn (1993) was not robust enough.

Further identification work to the study of Yu and Kobryn (1993) is provided in the following study by Yu (1993).

<b>Reference:</b>	<b>Yu, W. (1993)</b> Characterisation of Volatiles from Daminozide Aerobic Soil Metabolism
Report No.:	A.8.1.22
Document No:	-
Guideline:	-
GLP:	Yes
<b>Previous evaluation:</b>	In DAR (1999)

#### Material and methods:

<sup>14</sup>C-labeled and non-labeled chemicals were used in this study:

Test material: <sup>14</sup>C-methyl label daminozide

Specific radioactivity: 24.7 mCi/mmol

Radiochemical purity: > 98%

Sample/Batch ID: CSL-91-344-39-30

Test material: daminozide

CAS Number 1596-84-5

Purity: 99.6%

Lot # AC-1322-45

A previous study (project 92123) conducted on the aerobic soil metabolism of daminozide showed that daminozide degraded rapidly under the experimental conditions. At the 72-hour incubation interval, almost 60% of daminozide

was mineralised to carbon dioxide. In the ethylene glycol trap, which normally traps the neutral volatile compounds,  $^{14}\text{C}$ -radioactivity accounted for approximately 6.1% (equivalent to 0.59 ppm) of applied dose at the 16-hour sampling interval. While this level is relatively low, the agency has recommended that further characterisation should be attempted. Thus, this study was initiated to further investigate the contents in the ethylene glycol trap.

A number of experiments and analytical procedures have been performed on the characterisation of volatiles from the aerobic soil metabolism of daminozide. These include ion chromatography, chromotropic acid reaction, purge and trap/gas chromatography/mass spectrometry, headspace volatile/gas chromatography/mass spectrometry, as well as high-performance liquid chromatography. Neither N-nitrosodimethylamine (NDMA), unsymmetrical dimethylhydrazine (UDMH), nor dimethylamine (DMA) was detected in the ethylene glycol trap. HPLC analysis of the headspace volatiles and the ethylene glycol trap from various experiments appeared to show trace level of paraformaldehyde, which is likely to have been originated from formaldehyde.

### RMS comments and conclusion

Formaldehyde was probably wrongly identified and the metabolite observed in the study was probably methanol. Therefore, the analysis of the volatile in this study was also not correct, paraformaldehyde cannot be formed from formaldehyde, that was in fact methanol.

Statement provided by the notifier:

*Re-examination of the study report of Yu and Kobryn, 1993, in which the presence of formaldehyde was reported, demonstrates that the previous study only utilised HPLC analysis with radio- and UV detection. No confirmatory analytical method was reported and the only reference standard investigated was that for formaldehyde.*

*Therefore, though the formaldehyde standard eluted with a comparable retention time to the metabolite peak in the original study, because the peak was un-retained the degradation product observed in that study could be any polar compound which would also be likely to be un-retained, including methanol. Therefore, the method of analysis in the study of Yu and Kobryn, 1993, does not allow a robust identification of the metabolite observed.*

*The new study of Möndel, 2015 (presented below), is the definitive aerobic soil degradation study for daminozide. The only confirmed metabolite identification from soil degradation studies is for the metabolite M1 from this study, which is confirmed as methanol. It is therefore most likely, that the polar metabolite observed in the study of Yu and Kobryn, 1993, is also methanol and not formaldehyde as reported in that study report.*

<b>Reference:</b>	<b>Möndel, M. (2015)</b> Degradation of [ $^{14}\text{C}$ ]-Daminozide in four soils incubated under aerobic conditions at $20 \pm 2$ °C in the dark
Report No.:	AS358
Document No:	-
Guideline:	OECD Guideline 307; Aerobic and Anaerobic Transformation in Soil, April 24, 2002 OCSPP 835.4100 Aerobic Soil Metabolism (October, 2008)
GLP:	Yes
<b>Previous evaluation:</b>	Submitted for the purpose of renewal

<b>Reference:</b>	<b>Jones, M. (2015)</b> Daminozide: Analysis of Unknown Metabolites in Soil
Report No.:	SEL/8273/1
Document No:	-
Guideline:	None
GLP:	No
<b>Previous evaluation:</b>	Submitted for the purpose of renewal

**Test item:**

Test material:  $^{14}\text{C}$ -labelled daminozide

Specific radioactivity: 25 mCi/mmol

Radiochemical purity: > 98%

**Executive Summary:**

The route and rate of aerobic soil degradation of [ $^{14}\text{C}$ ]-daminozide was investigated in four soils incubated for 62 days in the dark at  $20 \pm 2^\circ\text{C}$  and 40% MWHC. [ $^{14}\text{C}$ ]-daminozide was applied at a concentration of 10.2 mg/kg dry soil, corresponding to a field application rate of 7.65 kg a.s./ha (assuming a soil mixing depth of 5 cm and a density of  $1.5 \text{ g/cm}^3$ ). Individual test vessels were ventilated at regular intervals with air, and the outgoing air was passed through a trapping system to retain  $\text{CO}_2$  (soda lime traps) and organic volatiles (quartz wool wetted with 2% paraffin in hexane).

Duplicate samples of each soil were collected immediately after application, at study termination at 62 days, and at 8 intermediate time-points. The soils were extracted at ambient temperature using two different extraction solvents: n-propanol/ $\text{H}_2\text{O}$  (72:28, v/v) + 1%  $\text{NH}_4\text{OH}$  up to four times until < 5% AR was extracted, and once with acetone. Radioactivity within individual extracts was analysed by LSC. After concentration (and for selected samples before concentration) the combined extracts were analysed by HPLC with radio- and UV-detection, and for selected samples additionally by TLC and LC/MS/MS. Unextracted residues were determined by combustion and LSC.

Mean sample recoveries were > 90% AR in three of the four soils, and 89.2% AR in the fourth soil (Soil III). All individual sample recoveries were >85% AR, with the sole exception of a single replicate 62 days after treatment in Soil III, for which clear losses of  $^{14}\text{CO}_2$  occurred. The low recovery in this sole replicate does not affect the validity of the test. High mineralisation of  $^{14}\text{C}$ -daminozide was observed in all four soils with  $^{14}\text{CO}_2$  increasing over time to reach 57.9 – 68.4% AR by the end of the study. Organic volatile compounds were always <0.1% AR.

Unextracted residues also increased to maximum concentrations of 26.7 to 41.0% AR, which decreased slightly by study termination to 23.1 to 33.5% AR.

Daminozide was observed to degrade rapidly from 87.5 – 99.5% AR (as determined when applied directly to the extraction solvent), and 84.7 – 91.6% AR following extraction from soil in the day 0 sample, to <0.1% AR in all soils after 7 days incubation.

Characterisation of soil extracts displayed only one very polar fraction (M1) at concentrations >5% AR. The maximum concentrations of M1 were 18.6 – 27.2% AR 1-2 days after application. Concentrations then declined rapidly such that final concentrations in all soils were  $\leq 2.4\%$  AR 34 DAT. No other fraction exceeded 0.3% AR.

Considerable efforts were made to identify the unknown metabolite M1 by multiple HPLC and LC/MS techniques, either directly or after derivatisation and/or sample clean-up. These attempts were not successful, and a definitive conclusion on the identity of the metabolite M1 was not possible. However, it was concluded that the unknown metabolite is highly polar, and has a high aqueous solubility. Targeting of dimethylamine, NDMA and UDMH demonstrated that the metabolite M1 does not correspond to those three compounds or any other hydrazine. Overall, because derivatisation with DNPH and LC/MS analyses could not exclude formaldehyde, and other HPLC analyses showed that M1 might also be methanol, these two compounds are considered to be the most likely identities of M1.

The degradation rates of daminozide and its metabolite M1 in soil were calculated according to FOCUS kinetics guidance (2006) using the software CAKE v. 2.0. SFO kinetics provided good fits for the degradation of both daminozide and M1 in all four soils. Daminozide degraded rapidly with  $DT_{50}$  values in all four soils of <0.4 days. M1 also degraded rapidly with  $DT_{50}$  values of 4.5 – 6.2 days.

## I. MATERIAL AND METHODS

The route and rate of aerobic soil degradation of [ $^{14}\text{C}$ ]-daminozide (specific radioactivity 25 mCi.mmol<sup>-1</sup>; >98% radiochemical purity) was investigated in four soils incubated for 62 days in the dark at  $20 \pm 2^\circ\text{C}$  and 40% MWHC. Characteristics of the soils are presented in Table B.8.1.1.1-23. Freshly collected soils were passed through a 2 mm sieve, and stored in a cooling chamber at  $1 - 10^\circ\text{C}$  until use 2-3 weeks after collection, when 100 g of each soil was dispensed into individual test vessels. Prior to application of the test item, soils were acclimated to  $20^\circ\text{C}$  and 40% MWHC. [ $^{14}\text{C}$ ]-daminozide was applied at a concentration of 10.2 mg/kg dry soil, corresponding to a field application rate of 7.65 kg a.s./ha (assuming a soil mixing depth of 5 cm and a density of 1.5 g/cm<sup>3</sup>). Individual test vessels were ventilated at regular intervals with air, and the outgoing air was passed through a trapping system to retain CO<sub>2</sub> (soda lime traps) and organic volatiles (quartz wool wetted with 2% paraffin in hexane).



**Table B.8.1.1.1-23: Composition of the four soils used in aerobic degradation tests**

Soil No.	I	II	III	IV
Soil Name	LUFA 2.4	LUFA 2.2	LUFA 5M	Fisli
Soil classification (USDA)	Loam	Loamy sand	Sandy loam	Silt loam
sand (50 µm – 2 mm)	33.2	76.5	57.8	10.4
silt (2 µm - 50 µm)	40.6	15.3	30.7	61.5
clay (<2 µm)	26.2	8.2	11.5	28.1
pH (0.01 M CaCl <sub>2</sub> )	7.2	5.5	7.3	6.8
CEC (meq/ 100 g)	32.2	10.2	17.1	26.0
Organic Carbon (%)	2.21	1.74	0.95	2.1
Water Holding Capacity (pF 2.0)	34.50%	--	--	42.3%
Maximum Water Holding Capacity (g/100g)	43.8	42.5	39.2	--
Bulk density (kg/dm <sup>3</sup> )	1.289	1.247	1.314	--
Microbial carbon [mg C <sub>mic</sub> /100 g DM]				
Day 0	43.30 / 41.74	33.44 / 35.18	38.44 / 36.78	42.86 / 42.96
Day 62 (with TI)	54.81 / 56.59	30.07 / 31.79	-- / 35.11	47.73 / 41.30
Day 62 (without TI)	48.21 / 48.32	30.06 / 31.78	31.81 / 35.12	37.95 / 44.59
Day 62 (with Solvent)	54.84 / 58.28	33.37 / 31.80	33.47 / 33.47	49.90 / 47.88

TI = test item

Duplicate soil samples of each soil were collected immediately after application, at study termination at 62 days, and at 8 intermediate time-points. The soils were extracted at ambient temperature using two different extraction solvents: n-propanol/H<sub>2</sub>O (72:28, v/v) + 1% NH<sub>4</sub>OH up to four times until <5% AR was extracted, and once with acetone. Radioactivity in individual extracts were analysed by LSC. N-propanol/H<sub>2</sub>O extracts were combined and concentrated by rotary evaporation at 40°C. The combined extracts (after concentration and for selected samples before concentration) were analysed by HPLC with radio- and UV-detection, and for selected samples additionally by TLC. It was observed that after 2 hours of incubation, the recovery of the concentrated extracts was below 90% and was due to the evaporation of metabolite M1. Due to the low amount of radioactivity in acetone extracts (≤1.3% AR) chromatographic analyses were not performed on them. Unextracted residues were determined by combustion and LSC.

Due to the expected rapid degradation of daminozide, the initial amount applied was determined by extracting untreated soil as described, and applying the test item to the soil extract, and analysing by LSC and HPLC. In addition, initial samples were extracted in two ways: for Time 0 samples the test item was applied to soil samples in extraction bottles already containing the extraction solvent. Extraction was performed for 1 minute, before

centrifugation. For Time 0A samples the test item was applied as for all samples and the first extraction then performed for 1 minute as described for Time 0 samples.

The re-applied soil samples from soil II were incubated for 4 hours and one sample was extracted with acetonitrile/H<sub>2</sub>O (72:28, v/v) + 1% NH<sub>4</sub>OH and one sample was extracted with n-propanol/H<sub>2</sub>O (72:28, v/v) + 1% NH<sub>4</sub>OH. The extracts were analysed by HPLC and TLC. A final extraction step was conducted using acetone as the extraction solvent to be able to dry out the soils for combustion.

The identification of [<sup>14</sup>C]-daminozide in the application solution as well as the identification of the parent in extracts was confirmed by LC-MS/MS. Additionally, reference items UDMH, dimethylamine and NDMA were analysed directly or after fortification of the extracts by HPLC and/or LC-MS/MS. Derivatisation techniques; with 2,4-dinitrophenylhydrazine (DNPH) to confirm the presence of formaldehyde, and with formaldehyde to confirm the presence of UDMH and other hydrazines, were performed. Additionally, aliquots of aqueous extracts were subjected to alkaline and acidic hydrolysis to release any conjugated metabolites.

Further characterisation of M1 was attempted in the study of Jones, 2015. The 72:28 acetonitrile: water + 1% Ammonia, and 72:28 isopropanol: water + 1% Ammonia extracts from the 4 hour samples were analysed by twelve LC-MS methods with UV and [<sup>14</sup>C]-detection. Throughout the investigations, mass spectral scanning was performed from m/z 20 upwards. In addition, a derivatisation technique to specifically target UDMH was investigated with one of the LC-MS methods, and the presence of UDMH was also investigated in the HILIC methods, by spiking the extract with 20 µg/mL UDMH reference standard, to assess whether its retention time matched that of the target metabolite. Multiple sample clean-up techniques, including several SPE and liquid-liquid partition methods, as well as a purge and trap method, were evaluated in an attempt to remove any matrix effects. GC/MS was also investigated but no evidence of either daminozide or the metabolite was observed in any of the runs.

The microbial biomass of the soils was determined prior to application and at study termination in soils either untreated or treated with non-radiolabelled test item or treated with the application solvent.

The degradation rate of daminozide and its metabolite M1 in soil was calculated according to FOCUS kinetics guidance (2006) using the software CAKE v. 2.0. For parent daminozide SFO and FOMC kinetics were both applied to the data. SFO kinetics alone were investigated for the metabolite M1, which was considered as part of a degradation scheme in which daminozide degraded to M1 and a sink compartment, and M1 degraded solely to a sink compartment. The suitability of the fit of the models was evaluated both visually, based on a graphical plot of the degradation and in a plot of the residuals, and statistically by calculating the minimum % error required to pass the  $\chi^2$  test at a probability of 0.05. For SFO kinetics a t-test was also performed to evaluate whether the determined parameters were significantly different to 0.

## II. RESULTS AND DISCUSSION

The recoveries of radioactivity from all four soils are presented in Table B.8.1.1.1.1-24 -

**Table B.8.1.1.1.1-27.** The majority of samples in soils I, II and IV display recoveries > 90% AR, and this is reflected in the mean sample recoveries all of which are > 90% AR. Where individual samples display recoveries < 90% AR, all are greater than 85% AR, and do not affect the validity of the study. For Soil III the mean recovery is 89.2% AR, however the majority of samples display recoveries > 85% AR. The sole exception to this is for a single replicate 62 days after treatment, and which was attributable to losses of  $^{14}\text{CO}_2$ . The low recovery in this sole replicate does not affect the validity of the test.

High mineralisation of  $^{14}\text{C}$ -daminozide was observed in all four soils with  $^{14}\text{CO}_2$  increasing over time to reach mean values 57.9-68.4% AR by the end of the study (NB. a single replicate in Soil III was disregarded because of the clear losses of  $^{14}\text{CO}_2$ ). Unextracted residues also increased to maximum concentrations of 26.7 to 41.0% AR, which decreased slightly by study termination to 23.1 to 33.5% AR.

The characterisation of radioactivity in the soil extracts is presented in



**Table B.8.1.1.1.1-28 - Table B.8.1.1.1.1-31.** The detection and quantification is based on results from HPLC analysis of combined and concentrated extracts. Daminozide was observed to degrade rapidly from 87.5 – 99.5% AR (as determined when applied directly to the extraction solvent), and 84.7 – 91.6% AR following extraction from soil in the day 0 sample, to <0.1% AR in all soils after 7 days incubation.

Soil extracts displayed only one very polar fraction (M1). After concentration significant reductions in the measured concentrations of M1 were observed. However, low recovery was obtained after concentration, and the losses corresponded to the amount of fraction M1. Consequently, the total amount of M1 was considered to be the sum of the amount detected in concentrated extracts and the amount of evaporated radioactivity after concentration of the extracts. The maximum concentrations of M1 were 18.6 – 27.2% AR 1-2 days after application. Concentrations then declined rapidly such that final concentrations in all soils were  $\leq 2.4\%$  AR 34 DAT. No other fraction exceeded 0.3% AR.

Significant efforts were made to identify the unknown M1 by HPLC retention time comparison to reference standards and by LC/MS, either directly or after derivatisation. However, these attempts were not successful due to the extremely high polarity of M1, and most probably, low molecular weight. Aliquots of extracts were derivatised with formaldehyde to investigate the presence of UDMH. However, no evidence that UDMH or other hydrazines were present was observed. Additionally, no clear ionisation or detection of the molecular masses in LC/MS analyses could be obtained. The results however have shown that this fraction does not correspond to the available reference standards UDMH, dimethylamine and NDMA. However, due to the very high polarity of these reference items and of M1 no well separation could be obtained. Hydrolysis under acidic or basic conditions did not show any changes of the retention time of M1, and showed that M1 is unlikely to be a conjugate.

Aliquots of extracts were derivatised with 2,4-dinitrophenylhydrazine (DNPH) to investigate the presence of formaldehyde. A similar but small UV peak which corresponded to the expected derivative was observed, however this peak was not observed in the radio-chromatogram. Additional LC/MS analysis of derivatised samples were neither able to confirm nor exclude the presence of formaldehyde due to the low sample concentration and presence of the soil matrix. It is likely that, if formed, formaldehyde will be present in the extract as paraformaldehyde.

HPLC analysis also demonstrated that M1 and methanol have similar retention times and therefore that M1 could also be methanol.

Of the twelve LC/MS methods investigated in the study of Jones, 2015, only the Hydrophilic Interaction Chromatography (HILIC) method gave significant resolution of the metabolite from parent daminozide at low pH. Reverse-phase LC techniques were not able to resolve the unknown polar component from parent daminozide, and both were un-retained at neutral and low-pH. Weak anion exchange chemistry was also not able to retain the target compound. Despite observation of a good signal for daminozide, there was no evidence of ionisation of the target compound in MS analyses.

All SPE methods retained little or no radioactivity, while liquid-liquid partition also displayed little or no transfer to organic solvents. Purge and trap methods were also ineffective with little or no transfer to the methanol trap. Therefore, all sample clean-up techniques attempted were unsuccessful. Based on these analyses it is concluded that the metabolite has a high aqueous/ acetonitrile solubility and is preferentially retained by this phase.

In additional work performed to specifically target UDMH the MS spectra following derivatisation displayed no distinctive ions indicative of the hydrazine derivative being present. Extracts spiked with UDMH reference standard investigated in the HILIC methods did not display a retention time match with the unknown, M1. Mass spectral analysis of the reference standard peak displayed a strong and discrete  $[M+H]^+$  protonated molecular ion of  $m/z$  61, however, no ionisation of the target compound was observed. Ammonia was added to the spiked extract to closer emulate the conditions found during extraction. This resulted in a significantly different mass spectral profile for the UDMH reference standard, which did not co-elute with the unknown peak.

The results of the kinetic evaluation for daminozide and M1 are presented in Table 8.1.1.1.1-32 – 8.1.1.1.1-33. Results for both SFO and FOMC kinetic fitting for daminozide are presented. In two soils chi-squared %-error values were marginally lower for FOMC fitting. In these two soils FOMC kinetics were considered most appropriate for

deriving end-points for comparison to persistence triggers. However, chi-squared %-error values and visual fits and plots of residuals remained very good for the SFO fits and consequently the SFO fits for daminozide in all four soils were considered the most appropriate for use in modelling. Daminozide degraded rapidly with  $DT_{50}$  values in all four soils  $<0.4$  days. The degradation of the metabolite M1 was also considered with SFO kinetics alone. Though some chi-squared error values were marginally higher than recommended values in FOCUS kinetics guidance, these values are not considered as strict cut-offs and visual fits and plots of residuals, as well as t-test parameters were all acceptable, indicating good and certain fits. M1 also degraded rapidly with  $DT_{50}$  values of 4.5 – 6.2 days.

**Table B.8.1.1.1-24: Distribution of radioactivity in soil I (%AR)**

Soil I (LUFA 2.4) loam		Radioactivity (% of applied) for Incubation Time in Hours (days)										
[% AR]	sample	0 (blank)	0A	2	4	6	24	2 (days)	7 (days)	14 (days)	34 (days)	62 (days)
Extractables* I	a	96.1	91.7	80.4	71.1	62.8	32.1	21.2	12.9	2.4	1.3	n.p.
	b	94.2	90.0	79.1	71.6	62.4	30.6	20.9	13.5	2.4	1.4	n.p.
	mean	<b>95.2</b>	<b>90.8</b>	<b>79.8</b>	<b>71.4</b>	<b>62.6</b>	<b>31.4</b>	<b>21.0</b>	<b>13.2</b>	<b>2.4</b>	<b>1.4</b>	<b>n.p.</b>
Extractables** II	a	0.1	0.1	0.2	0.4	0.7	1.0	1.0	0.5	0.3	0.2	n.p.
	b	0.1	0.2	0.2	0.4	0.7	1.0	1.0	0.6	0.3	0.2	n.p.
	mean	<b>0.1</b>	<b>0.2</b>	<b>0.2</b>	<b>0.4</b>	<b>0.7</b>	<b>1.0</b>	<b>1.0</b>	<b>0.5</b>	<b>0.3</b>	<b>0.2</b>	<b>n.p.</b>
Total Extractables (I + II)	a	96.2	91.8	80.6	71.5	63.6	33.2	22.2	13.4	2.7	1.6	n.p.
	b	94.4	90.1	79.2	72.0	63.1	31.5	21.8	14.1	2.7	1.6	n.p.
	mean	<b>95.3</b>	<b>91.0</b>	<b>79.9</b>	<b>71.7</b>	<b>63.3</b>	<b>32.3</b>	<b>22.0</b>	<b>13.7</b>	<b>2.7</b>	<b>1.6</b>	<b>n.p.</b>
<sup>14</sup> CO <sub>2</sub>	a	n.p.	n.p.	1.0	2.0	3.5	16.7	30.6	42.3	52.6	56.5	57.9
	b	n.p.	n.p.	0.9	2.2	3.6	16.7	30.6	43.6	52.5	53.2	57.9
	mean	<b>n.p.</b>	<b>n.p.</b>	<b>0.9</b>	<b>2.1</b>	<b>3.5</b>	<b>16.7</b>	<b>30.6</b>	<b>43.0</b>	<b>52.6</b>	<b>54.8</b>	<b>57.9</b>
Organic volatiles	a	n.p.	n.p.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
	b	n.p.	n.p.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
	mean	<b>n.p.</b>	<b>n.p.</b>	<b>&lt;0.1</b>	<b>&lt;0.1</b>	<b>&lt;0.1</b>	<b>&lt;0.1</b>	<b>&lt;0.1</b>	<b>&lt;0.1</b>	<b>&lt;0.1</b>	<b>&lt;0.1</b>	<b>&lt;0.1</b>
Non-Extractables	a	3.8	7.0	14.7	21.4	29.2	40.4	40.7	34.3	37.0	33.3	33.7
	b	4.1	7.0	15.2	21.1	29.4	41.7	40.1	33.6	37.7	33.3	33.4
	mean	<b>3.9</b>	<b>7.0</b>	<b>14.9</b>	<b>21.2</b>	<b>29.3</b>	<b>41.0</b>	<b>40.4</b>	<b>34.0</b>	<b>37.3</b>	<b>33.3</b>	<b>33.5</b>
Total	a	100.0	98.8	96.3	94.9	96.2	90.2	93.5	90.0	92.3	91.5	91.6
	b	98.5	97.1	95.3	95.2	96.1	90.0	92.5	91.3	92.9	88.1	91.3
	mean	<b>99.2</b>	<b>98.0</b>	<b>95.8</b>	<b>95.1</b>	<b>96.2</b>	<b>90.1</b>	<b>93.0</b>	<b>90.7</b>	<b>92.6</b>	<b>89.8</b>	<b>91.4</b>
<b>Mean ± SD</b>		<b>93.8 ± 3.5</b>										

\* Room temperature extraction using n-propanol/water (72:28, v/v) + 1% NH<sub>4</sub>OH

\*\* Room temperature extraction using acetone

a/b Duplicate samples

n.p. not performed

SD Standard deviation

Blank application to soil extract

A application to soil

**Table B.8.1.1.1-25: Distribution of radioactivity in soil II (%AR)**

Soil II (LUFA 2.2) loamy sand		Radioactivity (% of applied) for Incubation Time in Hours (Days)											
[% AR]	sample	0 (blank)	0A	2	4	Rep. 4	6	24	2 (days)	7 (days)	14 (days)	34 (days)	62 (days)
Extractables* I	a	100.1	99.0	62.6	52.1	50.4	40.2	28.2	24.5	14.4	4.0	2.4	n.p.
	b	99.8	98.1	64.8	51.0	53.0	42.9	28.9	24.5	13.8	4.0	2.4	n.p.
	mean	<b>100.0</b>	<b>98.5</b>	<b>63.7</b>	<b>51.5</b>	<b>51.7</b>	<b>41.6</b>	<b>28.5</b>	<b>24.5</b>	<b>14.1</b>	<b>4.0</b>	<b>2.4</b>	<b>n.p.</b>
Extractables** II	a	0.1	0.1	0.8	0.9	0.8	1.0	1.0	0.9	1.0	0.7	0.7	n.p.
	b	0.1	0.1	0.8	1.0	0.7	1.0	0.9	0.9	1.0	0.7	0.7	n.p.
	mean	<b>0.1</b>	<b>0.1</b>	<b>0.8</b>	<b>1.0</b>	<b>0.7</b>	<b>1.0</b>	<b>0.9</b>	<b>0.9</b>	<b>1.0</b>	<b>0.7</b>	<b>0.7</b>	<b>n.p.</b>
Total Extractables (I + II)	a	100.2	99.0	63.4	53.0	51.2	41.3	29.2	25.4	15.3	4.7	3.1	n.p.
	b	99.9	98.2	65.6	52.0	53.7	43.9	29.8	25.4	14.7	4.7	3.1	n.p.
	mean	<b>100.0</b>	<b>98.6</b>	<b>64.5</b>	<b>52.5</b>	<b>52.5</b>	<b>42.6</b>	<b>29.5</b>	<b>25.4</b>	<b>15.0</b>	<b>4.7</b>	<b>3.1</b>	<b>n.p.</b>
<sup>14</sup> CO <sub>2</sub>	a	n.p.	n.p.	15.6	20.5	17.8	29.5	37.6	44.1	45.4	61.4	66.1	68.0
	b	n.p.	n.p.	15.9	14.3	19.2	27.7	36.7	44.1	46.8	59.7	66.2	68.8
	mean	<b>n.p.</b>	<b>n.p.</b>	<b>15.8</b>	<b>17.4</b>	<b>18.5</b>	<b>28.6</b>	<b>37.1</b>	<b>44.1</b>	<b>46.1</b>	<b>60.5</b>	<b>66.2</b>	<b>68.4</b>
Organic volatiles	a	n.p.	n.p.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
	b	n.p.	n.p.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
	mean	<b>n.p.</b>	<b>n.p.</b>	<b>&lt;0.1</b>	<b>&lt;0.1</b>	<b>&lt;0.1</b>	<b>&lt;0.1</b>	<b>&lt;0.1</b>	<b>&lt;0.1</b>	<b>&lt;0.1</b>	<b>&lt;0.1</b>	<b>&lt;0.1</b>	<b>&lt;0.1</b>
Non- Extractables	a	0.7	1.3	15.2	18.8	18.9	22.2	23.0	23.7	24.3	26.8	23.5	23.3
	b	0.8	1.2	15.3	19.3	20.0	21.9	23.1	23.1	25.1	26.7	23.2	22.9
	mean	<b>0.8</b>	<b>1.2</b>	<b>15.3</b>	<b>19.0</b>	<b>19.5</b>	<b>22.0</b>	<b>23.1</b>	<b>23.4</b>	<b>24.7</b>	<b>26.7</b>	<b>23.3</b>	<b>23.1</b>
Total	a	100.9	100.3	94.3	92.3	87.9	93.0	89.8	93.2	85.0	92.8	92.7	91.4
	b	100.7	99.4	96.9	85.5	92.9	93.5	89.6	92.7	86.6	91.1	92.5	91.7
	mean	<b>100.8</b>	<b>99.9</b>	<b>95.6</b>	<b>88.9</b>	<b>90.4</b>	<b>93.3</b>	<b>89.7</b>	<b>92.9</b>	<b>85.8</b>	<b>92.0</b>	<b>92.6</b>	<b>91.5</b>
<b>Mean ± SD</b>		<b>92.0 ± 4.0</b>											

\* Room temperature extraction using n-propanol/water (72:28, v/v) + 1% NH<sub>4</sub>OH

\*\* Room temperature extraction using acetone

a/b Duplicate samples

n.p. not performed

SD Standard deviation

Blank application to soil extract

A application to soil

Rep. 4 Repeated samples. Replicate a was extracted with n-propanol/water (72:28, v/v) + 1% NH<sub>4</sub>OH and replicate b with acetonitrile/water (72:28, v/v) + 1% NH<sub>4</sub>OH



Table B.8.1.1.1-26: Distribution of radioactivity in soil III (%AR)

Soil III (LUFA 5M) sandy loam		Radioactivity (% of applied) for Incubation Time in Hours (Days)										
[% AR]	sample	0 (blank)	0A	2	4	6	24	2 (days)	7 (days)	14 (days)	34 (days)	62 (days)
Extractables* I	a	95.9	94.8	65.8	59.0	43.9	24.6	21.2	10.6	3.7	2.0	n.p.
	b	96.3	97.7	64.4	59.4	40.5	24.3	21.0	10.9	3.8	2.1	n.p.
	mean	<b>96.1</b>	<b>96.2</b>	<b>65.1</b>	<b>59.2</b>	<b>42.2</b>	<b>24.4</b>	<b>21.1</b>	<b>10.7</b>	<b>3.7</b>	<b>2.0</b>	<b>n.p.</b>
Extractables** II	a	0.1	0.1	0.8	0.8	1.2	1.1	1.0	0.8	0.6	0.5	n.p.
	b	0.1	0.1	0.7	0.9	1.3	1.2	1.0	0.8	0.6	0.5	n.p.
	mean	<b>0.1</b>	<b>0.1</b>	<b>0.8</b>	<b>0.8</b>	<b>1.2</b>	<b>1.2</b>	<b>1.0</b>	<b>0.8</b>	<b>0.6</b>	<b>0.5</b>	<b>n.p.</b>
Total Extractables (I + II)	a	96.0	94.9	66.6	59.8	45.1	25.7	22.2	11.4	4.3	2.5	n.p.
	b	96.4	97.8	65.1	60.3	41.8	25.5	22.0	11.6	4.3	2.6	n.p.
	mean	<b>96.2</b>	<b>96.3</b>	<b>65.9</b>	<b>60.1</b>	<b>43.5</b>	<b>25.6</b>	<b>22.1</b>	<b>11.5</b>	<b>4.3</b>	<b>2.6</b>	<b>n.p.</b>
<sup>14</sup> CO <sub>2</sub>	a	n.p.	n.p.	4.9	6.9	13.8	29.3	37.2	40.9	50.1	57.7	62.1
	b	n.p.	n.p.	5.9	6.8	15.3	30.0	38.0	44.0	52.8	58.4	38.4***
	mean	<b>n.p.</b>	<b>n.p.</b>	<b>5.4</b>	<b>6.8</b>	<b>14.6</b>	<b>29.6</b>	<b>37.6</b>	<b>42.4</b>	<b>51.4</b>	<b>58.1</b>	<b>50.2</b>
Organic volatiles	a	n.p.	n.p.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
	b	n.p.	n.p.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
	mean	<b>n.p.</b>	<b>n.p.</b>	<b>&lt;0.1</b>	<b>&lt;0.1</b>	<b>&lt;0.1</b>	<b>&lt;0.1</b>	<b>&lt;0.1</b>	<b>&lt;0.1</b>	<b>&lt;0.1</b>	<b>&lt;0.1</b>	<b>&lt;0.1</b>
Non- Extractables	a	2.7	2.6	18.9	22.5	29.3	30.2	30.1	31.6	32.5	28.6	28.1
	b	1.8	2.5	19.7	22.5	29.7	30.6	30.7	31.6	32.2	28.2	27.2
	mean	<b>2.2</b>	<b>2.5</b>	<b>19.3</b>	<b>22.5</b>	<b>29.5</b>	<b>30.4</b>	<b>30.4</b>	<b>31.6</b>	<b>32.3</b>	<b>28.4</b>	<b>27.6</b>
Total	a	98.7	97.5	90.3	89.2	88.2	85.2	89.6	83.9	86.8	88.8	90.1
	b	98.2	100.3	90.8	89.6	86.8	86.1	90.8	87.2	89.3	89.2	65.6
	mean	<b>98.4</b>	<b>98.9</b>	<b>90.6</b>	<b>89.4</b>	<b>87.5</b>	<b>85.6</b>	<b>90.2</b>	<b>85.6</b>	<b>88.1</b>	<b>89.0</b>	<b>77.9</b>
<b>Mean ± SD</b>		<b>89.2 ± 6.5</b>										

\* Room temperature extraction using n-propanol/water (72:28, v/v) + 1% NH<sub>4</sub>OH

\*\* Room temperature extraction using acetone

\*\*\* The lower result of repetition b was due to losses of <sup>14</sup>CO<sub>2</sub> during sampling

a/b Duplicate samples

n.p. not performed

SD Standard deviation

Blank application to soil extract

A application to soil

**Table B.8.1.1.1-27: Distribution of radioactivity in soil IV (%AR)**

Soil IV (Fislis) silt loam		Radioactivity (% of applied) for Incubation Time in Hours (Days)										
[% AR]	sample	0 (blank)	0A	2	4	6	24	2 (days)	7 (days)	14 (days)	34 (days)	62 (days)
Extractables* I	a	92.7	93.3	62.0	58.7	41.7	25.1	21.6	9.5	3.1	1.6	n.p.
	b	92.7	92.8	62.7	59.0	42.8	25.2	21.3	9.5	2.8	1.6	n.p.
	mean	<b>92.7</b>	<b>93.1</b>	<b>62.4</b>	<b>58.9</b>	<b>42.2</b>	<b>25.1</b>	<b>21.4</b>	<b>9.5</b>	<b>2.9</b>	<b>1.6</b>	<b>n.p.</b>
Extractables** II	a	0.2	0.2	0.7	1.0	1.3	1.3	1.2	0.8	0.7	0.6	n.p.
	b	0.2	0.2	0.7	1.0	1.2	1.3	1.2	0.9	0.7	0.5	n.p.
	mean	<b>0.2</b>	<b>0.2</b>	<b>0.7</b>	<b>1.0</b>	<b>1.2</b>	<b>1.3</b>	<b>1.2</b>	<b>0.9</b>	<b>0.7</b>	<b>0.6</b>	<b>n.p.</b>
Total Extractables (I + II)	a	92.9	93.5	62.8	59.7	43.0	26.4	22.7	10.3	3.1	2.1	n.p.
	b	92.9	93.0	63.4	60.0	43.9	26.5	22.4	10.4	3.4	2.1	n.p.
	mean	<b>92.9</b>	<b>93.2</b>	<b>63.1</b>	<b>59.8</b>	<b>43.5</b>	<b>26.4</b>	<b>22.6</b>	<b>10.4</b>	<b>3.3</b>	<b>2.1</b>	<b>n.p.</b>
<sup>14</sup> CO <sub>2</sub>	a	n.p.	n.p.	4.4	5.7	18.4	32.7	40.5	49.2	55.7	63.0	64.2
	b	n.p.	n.p.	4.7	5.9	16.8	32.7	39.5	44.2	56.6	60.4	62.0
	mean	<b>n.p.</b>	<b>n.p.</b>	<b>4.6</b>	<b>5.8</b>	<b>17.6</b>	<b>32.7</b>	<b>40.0</b>	<b>46.7</b>	<b>56.1</b>	<b>61.7</b>	<b>63.1</b>
Organic volatiles	a	n.p.	n.p.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
	b	n.p.	n.p.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
	mean	<b>n.p.</b>	<b>n.p.</b>	<b>&lt;0.1</b>	<b>&lt;0.1</b>	<b>&lt;0.1</b>	<b>&lt;0.1</b>	<b>&lt;0.1</b>	<b>&lt;0.1</b>	<b>&lt;0.1</b>	<b>&lt;0.1</b>	<b>&lt;0.1</b>
Non- Extractables	a	5.6	5.8	22.1	23.9	29.7	28.6	27.0	29.8	32.7	28.0	28.3
	b	5.6	7.0	21.4	24.1	29.1	29.1	27.5	30.3	31.5	28.3	29.0
	mean	<b>5.6</b>	<b>6.4</b>	<b>21.8</b>	<b>24.0</b>	<b>29.4</b>	<b>28.8</b>	<b>27.2</b>	<b>30.1</b>	<b>32.1</b>	<b>28.2</b>	<b>28.6</b>
Total	a	98.6	99.3	89.3	89.4	91.1	87.7	90.2	89.3	91.4	92.0	92.5
	b	98.5	100.0	89.6	90.0	89.8	88.2	89.5	84.9	92.0	91.1	91.0
	mean	<b>98.6</b>	<b>99.6</b>	<b>89.4</b>	<b>89.7</b>	<b>90.5</b>	<b>88.0</b>	<b>89.8</b>	<b>87.1</b>	<b>91.7</b>	<b>91.5</b>	<b>91.8</b>
<b>Mean ± SD</b>		<b>91.6 ± 4.4</b>										

\* Room temperature extraction using n-propanol/water (72:28, v/v) + 1% NH<sub>4</sub>OH

\*\* Room temperature extraction using acetone

a/b Duplicate samples

n.p. not performed

SD Standard deviation

Blank application to soil extract

A application to soil

Table B.8.1.1.1-28: Characterisation of extracted radioactivity in Soil I (% AR)

Soil I (LUFA 2.4) loam		Radioactivity (% of applied) for Incubation Time in Hours (Days)									
[% AR]	sample	0 (blank)	0A	2	4	6	24	2 (days)	7 (days)	14 (days)**	34 (days)**
Daminozide	a	93.3	87.8	77.0	64.7	55.2	14.9	2.7	<0.1	n.p.	n.p.
	b	92.5	81.7	78.1	61.9	55.9	13.9	2.1	<0.1	n.p.	n.p.
	<b>mean</b>	<b>92.9</b>	<b>84.7</b>	<b>77.5</b>	<b>63.3</b>	<b>55.6</b>	<b>14.4</b>	<b>2.4</b>	<b>&lt;0.1</b>	<b>n.p.</b>	<b>n.p.</b>
M1* (RT: 3.7-4.8)	a	<0.1	<0.1	<0.1	<0.1	<0.1	0.4	1.0	1.6	n.p.	n.p.
	b	<0.1	<0.1	<0.1	<0.1	<0.1	0.7	1.5	2.1	n.p.	n.p.
	<b>mean</b>	<b>&lt;0.1</b>	<b>&lt;0.1</b>	<b>&lt;0.1</b>	<b>&lt;0.1</b>	<b>&lt;0.1</b>	<b>0.6</b>	<b>1.2</b>	<b>1.9</b>	<b>n.p.</b>	<b>n.p.</b>
Evaporation losses (M1)*	a	2.9	3.9	3.5	6.4	7.6	16.8	17.5	11.3	2.4	1.3
	b	1.7	8.3	1.0	9.7	6.5	15.9	17.3	11.4	2.4	1.4
	<b>mean</b>	<b>2.3</b>	<b>6.1</b>	<b>2.2</b>	<b>8.1</b>	<b>7.0</b>	<b>16.4</b>	<b>17.4</b>	<b>11.3</b>	<b>2.4</b>	<b>1.4</b>
M1 (total)	a	2.9	3.9	3.5	6.4	7.6	17.2	18.5	12.9	2.4	1.3
	b	1.7	8.3	1.0	9.7	6.5	16.6	18.8	13.5	2.4	1.4
	<b>mean</b>	<b>2.3</b>	<b>6.1</b>	<b>2.2</b>	<b>8.1</b>	<b>7.0</b>	<b>16.9</b>	<b>18.6</b>	<b>13.2</b>	<b>2.4</b>	<b>1.4</b>

a/b Duplicate samples

n.p. not performed

Blank application to soil extract

A application to soil

RT Retention time in minutes

\* HPLC-chromatograms of non-concentrated extracts showed a distinct peak M1 (retention time between 3.7-4.8 minutes). LSC-measurements of extracts before and after sample concentration showed that the amount of losses after concentration corresponded to the HPLC peak amount of M1 in extracts before concentration.

\*\* Due to low amount extracted no HPLC analysis was conducted. Extracted radioactivity was assigned to M1.

Table B.8.1.1.1-29: Characterisation of extracted radioactivity in Soil II (% AR)

Soil II (LUFA 2.2) loamy sand		Radioactivity (% of applied) for Incubation Time in Hours (Days)										
[% AR]	sample	0 (blank)	0A	2	4	Rep. 4	6	24	2 (days)	7 (days)	14 (days)**	34 (days)**
Daminozide	a	97.2	90.9	47.8	32.7	32.0	26.0	2.0	0.9	<0.1	n.p.	n.p.
	b	101.8	92.3	51.5	30.7	34.3	26.1	0.8	0.5	<0.1	n.p.	n.p.
	mean	<b>99.5</b>	<b>91.6</b>	<b>49.7</b>	<b>31.7</b>	<b>33.1</b>	<b>26.0</b>	<b>1.4</b>	<b>0.7</b>	<b>&lt;0.1</b>	<b>n.p.</b>	<b>n.p.</b>
M1* RT: 3.7-4.8	a	<0.1	<0.1	<0.1	<0.1	18.4***	<0.1	2.5	2.0	<0.1	n.p.	n.p.
	b	<0.1	<0.1	<0.1	<0.1	18.7***	<0.1	3.6	2.8	4.8	n.p.	n.p.
	mean	<b>&lt;0.1</b>	<b>&lt;0.1</b>	<b>&lt;0.1</b>	<b>&lt;0.1</b>	<b>18.6***</b>	<b>&lt;0.1</b>	<b>3.0</b>	<b>2.4</b>	<b>2.4</b>	<b>n.p.</b>	<b>n.p.</b>
Evaporation losses (M1)*	a	2.9	8.1	14.7	19.3	--	14.3	23.7	21.6	11.9	4.0	2.4
	b	<0.1	5.8	13.3	20.3	--	16.8	24.5	21.2	9.0	4.0	2.4
	mean	<b>1.4</b>	<b>7.0</b>	<b>14.0</b>	<b>19.8</b>	<b>--</b>	<b>15.5</b>	<b>24.1</b>	<b>21.4</b>	<b>10.5</b>	<b>4.0</b>	<b>2.4</b>
M1 (total)	a	2.9	8.1	14.7	19.3	18.4	14.3	26.2	23.6	11.9	4.0	2.4
	b	<0.1	5.8	13.3	20.3	18.7	16.8	28.1	24.0	13.8	4.0	2.4
	mean	<b>1.4</b>	<b>7.0</b>	<b>14.0</b>	<b>19.8</b>	<b>18.6</b>	<b>15.5</b>	<b>27.2</b>	<b>23.8</b>	<b>12.8</b>	<b>4.0</b>	<b>2.4</b>

a/b Duplicate samples

n.p. not performed

Blank application to soil extract

A application to soil

RT Retention time in minutes

\* HPLC-chromatograms of non-concentrated extracts showed a distinct peak M1 (retention time between 3.7-4.8 minutes). LSC-measurements of extracts before and after sample concentration showed that the amount of losses after concentration corresponded to the HPLC peak amount of M1 in extracts before concentration.

\*\* Due to low amount extracted no HPLC analysis was conducted. Extracted radioactivity was assigned to M1.

\*\*\* Sample analysed directly

Table B.8.1.1.1-30: Characterisation of extracted radioactivity in Soil III (% AR)

Soil III (LUFA 5M) sandy loam		Radioactivity (% of applied) for Incubation Time in Hours (Days)									
[% AR]	sample	0 (blank)	0A	2	4	6	24	2 (days)	7 (days)	14 (days)**	34 (days)**
Daminozide	a	94.5	85.8	57.3	44.4	30.8	1.6	0.2	<0.1	n.p.	n.p.
	b	97.0	91.3	53.7	46.7	25.2	3.1	0.3	<0.1	n.p.	n.p.
	mean	95.7	88.5	55.5	45.5	28.0	2.3	0.2	<0.1	n.p.	n.p.
M1* (RT: 3.7-4.8)	a	<0.1	<0.1	<0.1	<0.1	<0.1	2.7	2.2	3.2	n.p.	n.p.
	b	<0.1	<0.1	<0.1	<0.1	<0.1	2.6	1.9	3.6	n.p.	n.p.
	mean	<0.1	<0.1	<0.1	<0.1	<0.1	2.6	2.0	3.4	n.p.	n.p.
M2 (RT: 32.9-35.3)	a	<0.1	<0.1	<0.1	<0.1	<0.1	0.4	0.2	<0.1	<0.1	<0.1
	b	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.3	<0.1	<0.1	<0.1
	mean	<0.1	<0.1	<0.1	<0.1	<0.1	0.2	0.3	<0.1	<0.1	<0.1
Evaporation losses (M1)*	a	1.4	9.0	8.5	14.7	13.1	19.8	18.6	7.4	3.7	2.0
	b	<0.1	6.5	10.7	12.8	15.3	18.7	18.5	7.3	3.8	2.1
	mean	0.7	7.7	9.6	13.7	14.2	19.3	18.5	7.4	3.7	2.0
M1 (total)	a	1.4	9.0	8.5	14.7	13.1	22.6	20.8	10.6	3.7	2.0
	b	<0.1	6.5	10.7	12.8	15.3	21.3	20.4	10.9	3.8	2.1
	mean	0.7	7.7	9.6	13.7	14.2	21.9	20.6	10.7	3.7	2.0

a/b Duplicate samples

n.p. not performed

Blank application to soil extract

A application to soil

RT Retention time in minutes

\* HPLC-chromatograms of non-concentrated extracts showed a distinct peak M1 (retention time between 3.7-4.8 minutes). LSC-measurements of extracts before and after sample concentration showed that the amount of losses after concentration corresponded to the HPLC peak amount of M1 in extracts before concentration.

\*\* Due to low amount extracted no HPLC analysis was conducted. Extracted radioactivity was assigned to M1.

Table B.8.1.1.1.1-31: Characterisation of extracted radioactivity in Soil IV (% AR)

Soil IV (Fislis) silt loam		Radioactivity (% of applied) for Incubation Time in Hours (Days)									
[% AR]	sample	0 (blank)	0A	2	4	6	24	2 (days)	7 (days)	14 (days)**	34 (days)**
Daminozide	a	87.2	87.6	52.0	44.6	27.8	0.8	0.4	<0.1	n.p.	n.p.
	b	87.9	90.3	51.5	46.7	28.5	1.8	0.4	<0.1	n.p.	n.p.
	mean	<b>87.5</b>	<b>89.0</b>	<b>51.8</b>	<b>45.7</b>	<b>28.1</b>	<b>1.3</b>	<b>0.4</b>	<b>&lt;0.1</b>	<b>n.p.</b>	<b>n.p.</b>
M1* (RT: 3.7-4.8)	a	<0.1	<0.1	<0.1	<0.1	<0.1	2.2	1.1	3.3	n.p.	n.p.
	b	<0.1	<0.1	<0.1	<0.1	<0.1	1.4	1.2	<0.1	n.p.	n.p.
	mean	<b>&lt;0.1</b>	<b>&lt;0.1</b>	<b>&lt;0.1</b>	<b>&lt;0.1</b>	<b>&lt;0.1</b>	<b>1.8</b>	<b>1.1</b>	<b>1.6</b>	<b>n.p.</b>	<b>n.p.</b>
Evaporation losses (M1)*	a	5.5	5.7	10.0	14.1	14.0	22.0	20.0	6.2	3.1	1.6
	b	4.8	2.5	11.2	12.3	14.3	21.9	19.7	7.2	2.8	1.6
	mean	<b>5.2</b>	<b>4.1</b>	<b>10.6</b>	<b>13.2</b>	<b>14.1</b>	<b>21.9</b>	<b>19.9</b>	<b>6.7</b>	<b>2.9</b>	<b>1.6</b>
M1 (total)	a	5.5	5.7	10.0	14.1	14.0	24.2	21.1	9.5	3.1	1.6
	b	4.8	2.5	11.2	12.3	14.3	23.3	20.9	7.2	2.8	1.6
	mean	<b>5.2</b>	<b>4.1</b>	<b>10.6</b>	<b>13.2</b>	<b>14.1</b>	<b>23.8</b>	<b>21.0</b>	<b>8.3</b>	<b>2.9</b>	<b>1.6</b>

a/b Duplicate samples

n.p. not performed

Blank application to soil extract

A application to soil

RT Retention time in minutes

\* HPLC-chromatograms of non-concentrated extracts showed a distinct peak M1 (retention time between 3.7-4.8 minutes). LSC-measurements of extracts before and after sample concentration showed that the amount of losses after concentration corresponded to the HPLC peak amount of M1 in extracts before concentration.

\*\* Due to low amount extracted no HPLC analysis was conducted. Extracted radioactivity was assigned to M1.

Table B.8.1.1.1.1-32: Summary of the results of the kinetic determinations for daminozide (SFO and FOMC) in aerobic soil degradation studies

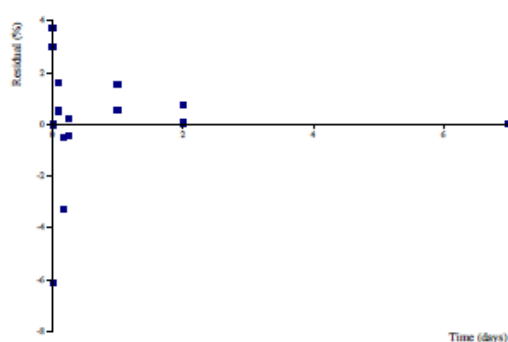
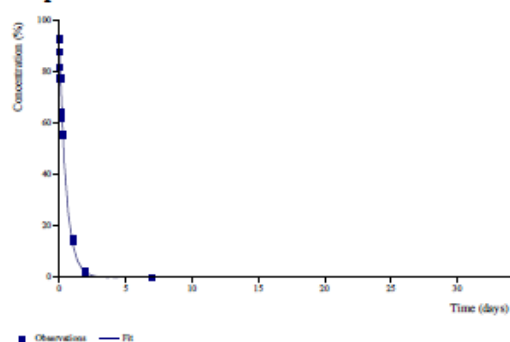
Soil	Kinetic model	$\chi^2$ error (%)	Kinetic parameters	DT <sub>50</sub> (d)	DT <sub>90</sub> (d)	Confidence interval (95%)		Prob. >t P value
						lower	upper	
LUFA 2.4 (loam)	SFO (modelling and persistence)	2.99	k = 1.90	0.37	1.21	-	-	6.0E-20
	FOMC	3.19	$\alpha = 2.97 \text{ E}3$ $\beta = 1.56 \text{ E}3$	0.37	1.21	$\alpha = 2.91\text{E}+03$ $\beta = 1.44\text{E}+03$	3.03E+03 1.68E+03	-
LUFA 2.2 (loamy sand)	SFO (modelling)	7.69	k = 6.56	0.11	0.35	-	-	9.8E-24
	FOMC (persistence)	4.15	$\alpha = 1.53$ $\beta = 0.15$	0.09	0.54	$\alpha = 0.9108$ $\beta = 0.06255$	2.155 0.245	-
LUFA 5M (sandy loam)	SFO (modelling)	6.03	k = 4.89	0.14	0.47	-	-	1.55E-22
	FOMC (persistence)	5.45	$\alpha = 2.62$ $\beta = 0.44$	0.13	0.62	$\alpha = 0.6109$ $\beta = 0.026$	4.625 0.849	-
FISLIS (silt loam)	SFO (modelling and persistence)	8.02	k = 4.62	0.15	0.50	-	-	1.26E-14
	FOMC	8.09	$\alpha = 3.43$ $\beta = 0.64$	0.14	0.61	$\alpha = -0.696$ $\beta = -0.2535$	7.556 1.528	-

**Table B.8.1.1.1-33: Summary of the results of the kinetic determinations for the metabolite M1 (SFO) in aerobic soil degradation studies**

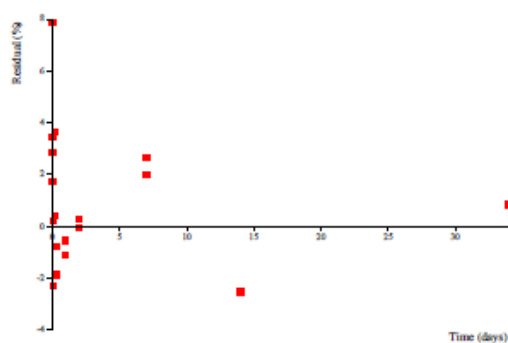
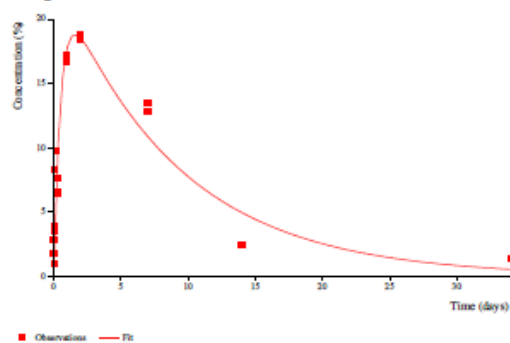
Soil	Kinetic model	$\chi^2$ error (%)	Kinetic parameters	DT <sub>50</sub> (d)	DT <sub>90</sub> (d)	Confidence interval	FF (from daminozide)	P
LUFA 2.4 (loam)	SFO/SFO	24.6	k = 0.113	6.2	20.5	nr	0.25	3.9E-6
LUFA 2.2 (loamy sand)	SFO/SFO	18.9	k = 0.115	6.1	20.1	nr	0.29	4.7E-26
LUFA 5M (sandy loam)	SFO/SFO	18.3	k = 0.118	5.9	19.4	nr	0.26	1.48E-12
FISLIS (silt loam)	SFO/SFO	18.3	k = 0.153	4.5	15.0	nr	0.29	8.09E-24

Nr - not reported

### Compartment Parent:

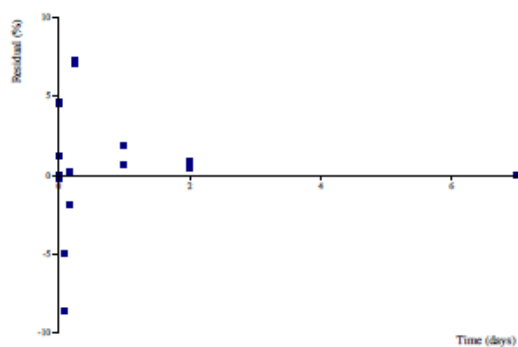
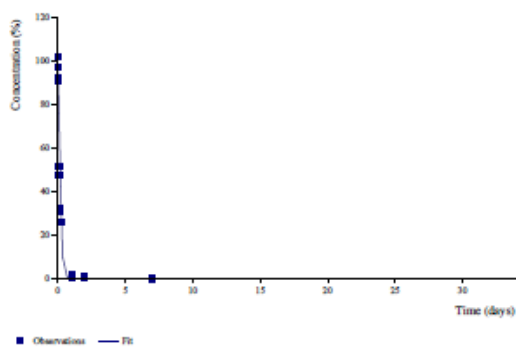
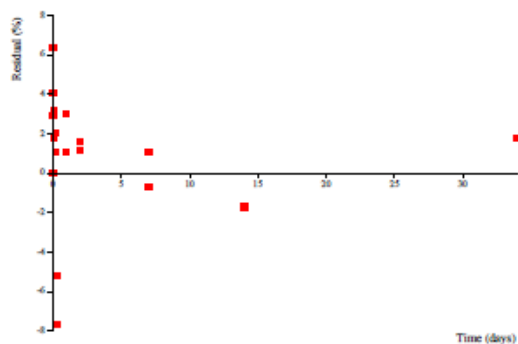
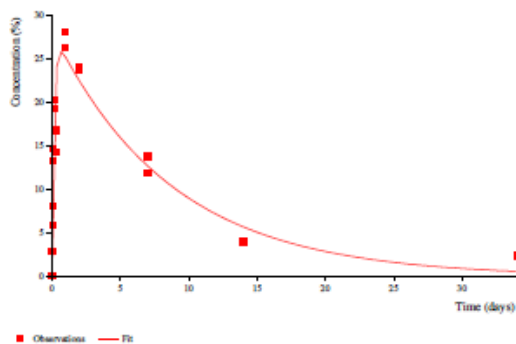


### Compartment A1:



Parent = daminozide  
A1 = M1 + evap. losses

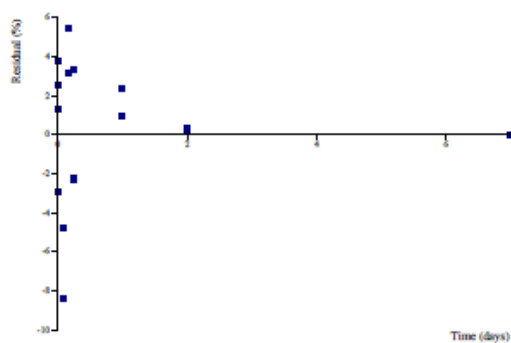
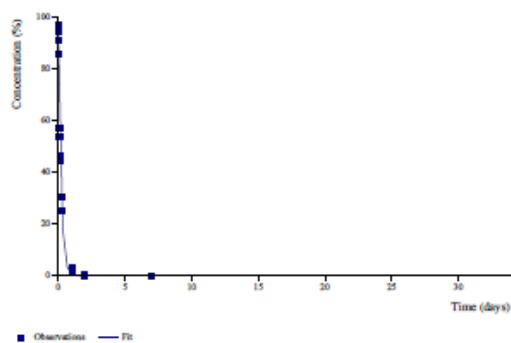
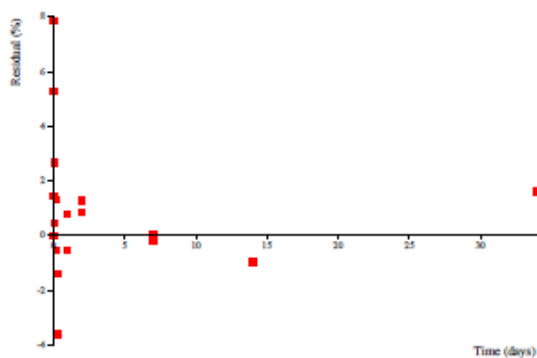
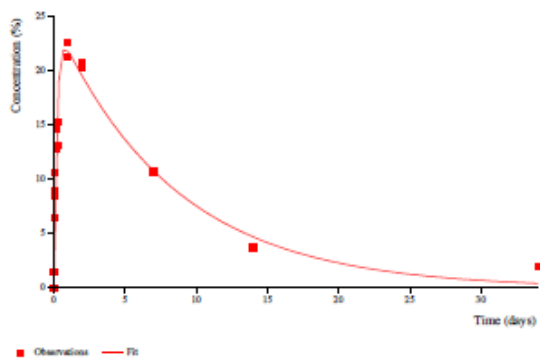
**Figure B.8.1.1.1-1: Graphical output of the kinetic evaluation of the data set for LUFA 2.4 soil (SFO)**

**Compartment Parent:****Compartment A1:**

Parent = daminozide

A1 = M1 + evap. losses

Figure B.8.1.1.1-2: Graphical output of the kinetic evaluation of the data set for LUFA 2.2 soil (SFO)

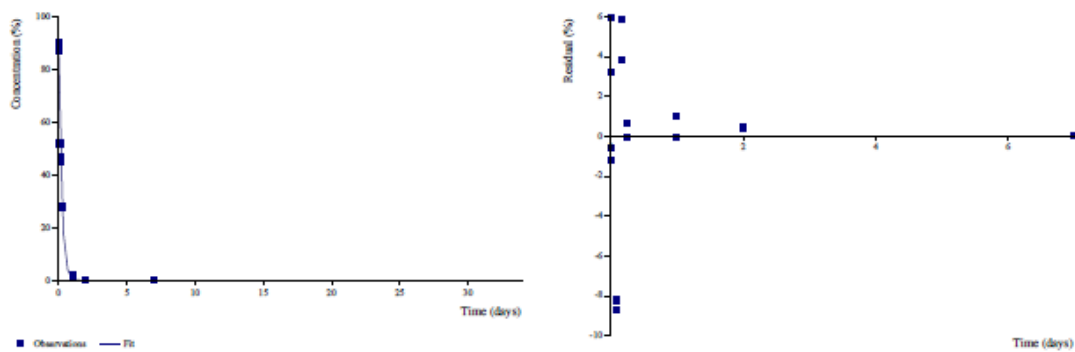
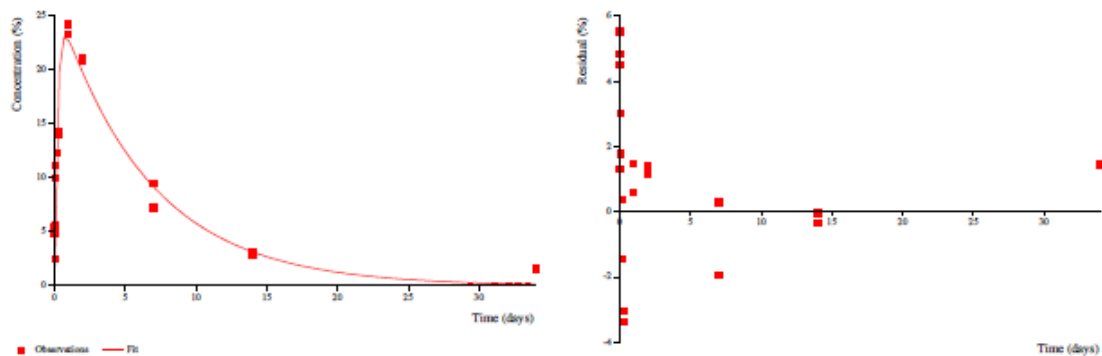
**Compartment Parent:****Compartment A1:**

Parent = daminozide

A1 = M1 + evap. losses

**Figure B.8.1.1.1-3: Graphical output of the kinetic evaluation of the data set for LUFA 5M soil (SFO)**

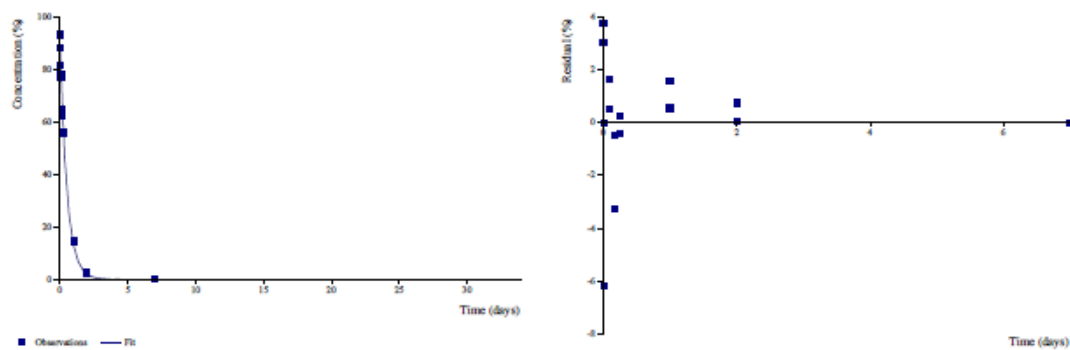
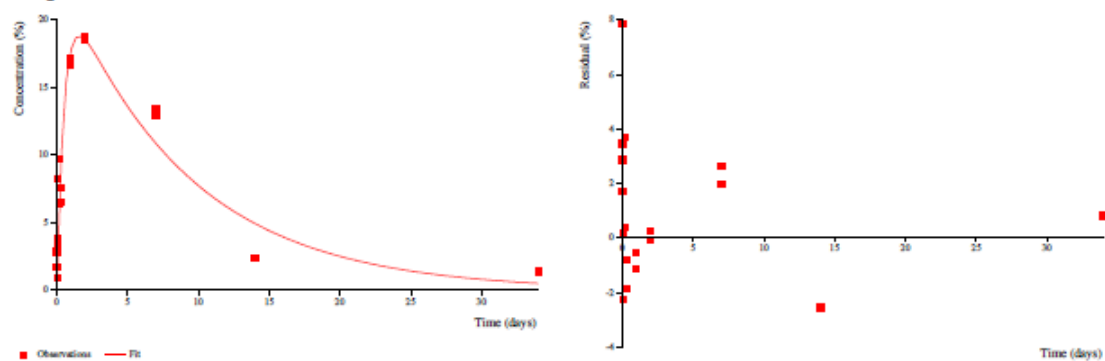


**Compartment Parent:****Compartment A1:**

Parent = daminozide

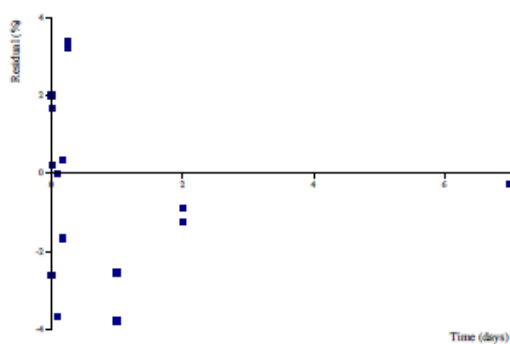
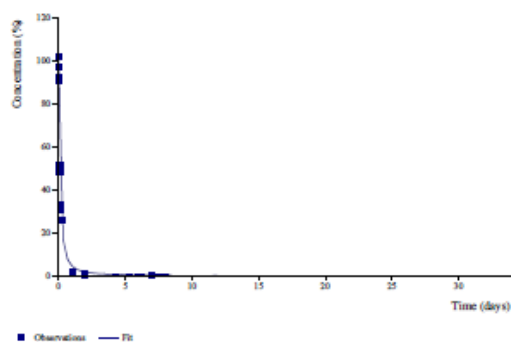
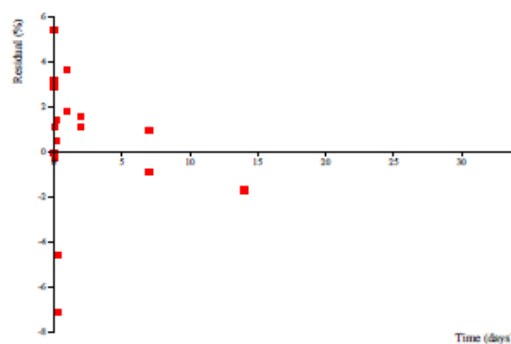
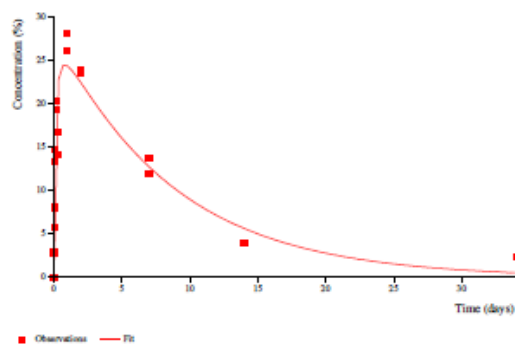
A1 = M1 + evap. losses

**Figure B.8.1.1.1-4: Graphical output of the kinetic evaluation of the data set for FISLIS soil (SFO)**

**Compartment Parent:****Compartment A1:**

Parent = daminozide  
A1 = M1 + evap. losses

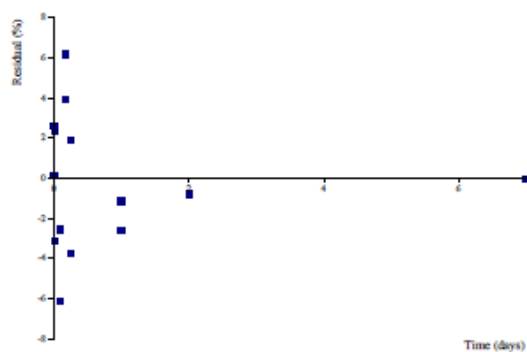
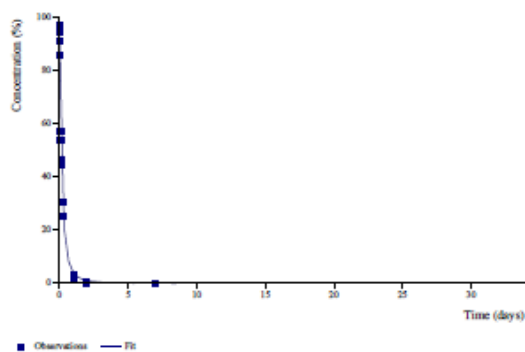
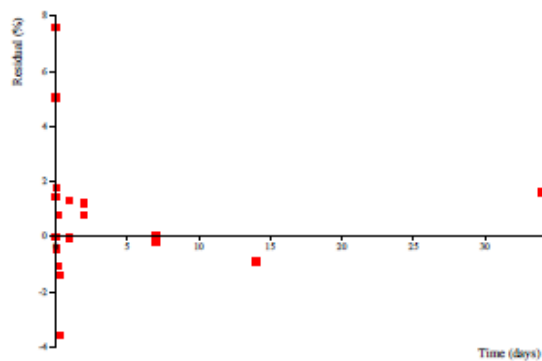
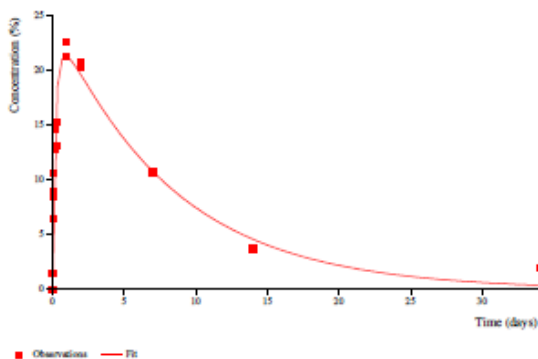
**Figure B.8.1.1.1-5: Graphical output of the kinetic evaluation of the data set for LUFA 2.4 soil (FOMC)**

**Compartment Parent:****Compartment A1:**

Parent = daminozide

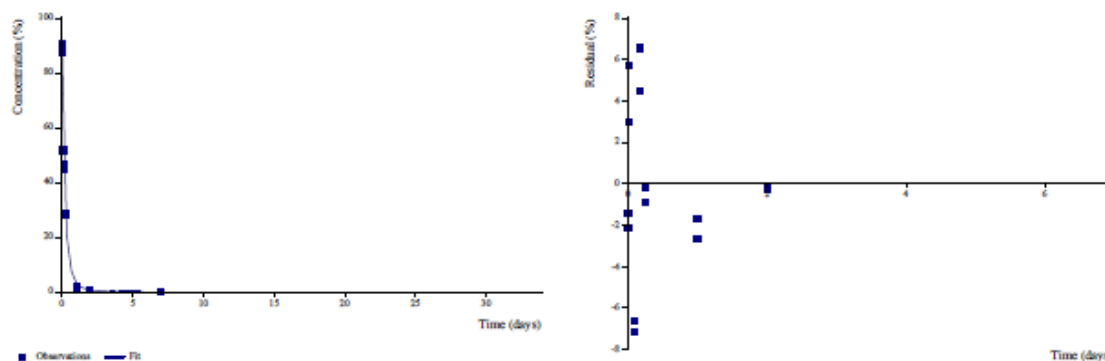
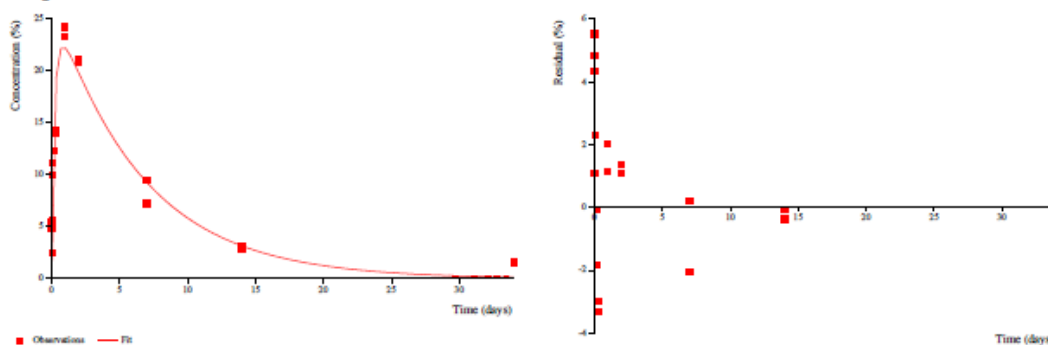
A1 = M1 + evap. losses

**Figure B.8.1.1.1-6: Graphical output of the kinetic evaluation of the data set for LUFA 2.2 soil (FOMC)**

**Compartment Parent:****Compartment A1:**

Parent = daminozide  
A1 = M1 + evap. losses

**Figure B.8.1.1.1-7: Graphical output of the kinetic evaluation of the data set for LUFA 5M soil (FOMC)**

**Compartment Parent:****Compartment A1:**

Parent = daminozide

A1 = M1 + evap. losses

**Figure B.8.1.1.1-8: Graphical output of the kinetic evaluation of the data set for FISLIS soil (FOMC)**

### III. CONCLUSION

Daminozide degraded rapidly, with SFO  $DT_{50}$  values of 0.1 – 0.4 days, in four aerobic soils incubated in the laboratory in the dark at 20°C and a soil moisture content of 40% MWHC.  $CO_2$  was shown to be the terminal degradation product, with maximum concentrations of 57.9 - 68.4% AR by the end of the study. Organic volatile compounds were always <0.1% AR. Unextracted residues also increased to maximum concentrations of 26.7 to 41.0% AR, which decreased slightly by study termination to 23.1 to 33.5% AR.

Daminozide degraded via one very polar fraction (M1) at concentrations >5% AR. The maximum concentrations of M1 were 18.6 – 27.2% AR, observed 1-2 days after application. Concentrations then declined rapidly ( $DT_{50}$  values of 4.5 – 6.2 days) such that final concentrations in all soils were  $\leq 2.4\%$  AR 34 DAT. No other fraction exceeded 0.3% AR. Considerable efforts were made to identify the unknown metabolite M1 by HPLC and LC/MS, either directly or after derivatisation and/or sample clean-up. These attempts were not successful, and a definitive conclusion on the identity of the metabolite M1 was not possible. However, it was concluded that the unknown

metabolite is highly polar, is volatile, and has a high aqueous solubility. Targeting of dimethylamine, NDMA and UDMH demonstrated that the metabolite M1 does not correspond to any of these compounds or any other hydrazine. Overall, because derivatisation and LC/MS analyses could not exclude formaldehyde, and other HPLC analyses showed that M1 might also be methanol, these two compounds are considered to be the most likely identities of M1.

### RMS comments and conclusion

Study was conducted according to OECD guideline 307 and is considered acceptable. Total recovery for Soil III was below recommended range for majority of sampling points but above 85% except the last sampling point where total recovery was 77.9% AR only due to CO<sub>2</sub> losses.

The RMS agrees with the modelling and persistence endpoints for parent daminozide. SFO kinetic fit is visually and statistically acceptable. The RMS is of opinion that FOMC for LUFA 2.2 and LUFA 5M soils gave slightly lower  $\chi^2$  error value but did not improve the fit visually. Therefore, SFO kinetics is acceptable for persistence and modelling endpoints for all soils. SFO kinetic fitting for metabolite M1 is considered appropriate by the RMS.

A further attempt to characterise the metabolite M1 in the soil extracts was made in the study of DeMaio, 2015, report is presented below.

<b>Reference:</b>	<b>DeMaio, W. (2015)</b> Degradation of [ <sup>14</sup> C]-Daminozide in Four Soils Incubated Under Aerobic Conditions at 20 ± 2 °C in the Dark: Characterization of a Daminozide
Report No.:	033709-1
Document No:	-
Guideline:	None
GLP:	No
<b>Previous evaluation:</b>	Submitted for the purpose of renewal

### Executive Summary:

Extracts of soil samples treated with [<sup>14</sup>C]-Daminozide from the study of Möndel, 2015, were analysed to determine the identity of the unknown polar metabolite, M1. Analysis of soil extracts by LC/TOF-MS analysis with comparison to reference standards confirmed that the polar metabolite was not UDMH, dimethylamine or NDMA. Derivatisation with DNPH demonstrated that trace amounts of formaldehyde were present in the soil extracts when analysed by LC/TOF-MS (Time Of Flight – MS). However, the minor amounts observed did not correspond to more than a small fraction of the total concentration of the peak, and when analysed with a radioactive detector, no response was observed. Consequently, it was concluded that the trace amounts of formaldehyde observed in LC/TOF-MS corresponded to natural background concentrations of formaldehyde in soil and not to the unidentified metabolite. Further work was performed with ion exclusion HPLC with LC-Refractive Index (RI) detection. The unknown radioactive peak was retained and was identified as methanol by comparison to reference standards for candidate compounds, co-chromatography with methanol fortified extracts and refractive index and radioactivity flow detection in series. The other remaining metabolite candidates, formaldehyde and formic acid, were excluded on the basis of comparison of the extract peak to reference standard retention times.

Consequently, it was concluded that the metabolite M1 from the aerobic soil degradation study of Möndel, 2015, was methanol.

## I. MATERIAL AND METHODS

Extracts of soil samples treated with [ $^{14}\text{C}$ ]-Daminozide from the study of Möndel, 2015, were analysed to determine the identity of the unknown polar metabolite, M1. Four extracts from the study of Möndel, 2015 were utilized in the analysis. The extract details are presented in Table B.8.1.1.1.1-34.

**Table B.8.1.1.1-34: Daminozide Soil Extracts Analysed**

	<b>Extract 1</b>	<b>Extract 2</b>	<b>Extract 3</b>	<b>Extract 4</b>
Sample	<b>4 hours</b> (1-fold appl. rate)	<b>4 hours</b> (1-fold appl. rate)	<b>24 hours</b> (1-fold appl. rate)	<b>24 hours</b> (10-fold appl. rate)
Application Rate ( $\mu\text{g/g}$ dry soil)	10.2	10.2	10.0	100.2
Concentration ( $\mu\text{g/L}$ parent equivalent)	4007.8	4208.8	2730	29,000
Amount (delivered) (mL)	46.2	59.2	19.4	11.5
Extraction Solvent	n-propanol /H <sub>2</sub> O (72:28, v/v) + 1% NH <sub>4</sub> OH	Acetonitrile/H <sub>2</sub> O (72:28, v/v) + 1% NH <sub>4</sub> OH	H <sub>2</sub> O	H <sub>2</sub> O
Radioactivity (dpm/mL)	467,019	483,416	38,356	415,278
Radioactivity (kBq)	372.1	500.6	12.4	79.6
Total Amount of Radioactivity (kBq)	964.7			

Soil extracts were initially analysed without further sample preparation by LC/TOF-MS analysis with two different solvent gradients and a Zorbax 300-SCX column (150  $\times$  4.6 mm, 5  $\mu\text{m}$ ). A TripleTOF 5600 mass spectrometer was used for detection, as well as radioactivity flow detection and UV/Vis detection for selected samples. Retention times and masses of the peak observed in the extracts were compared to reference standards for daminozide, UDMH, dimethylamine and NDMA.

Derivatisation of Extract 4 with 2,4-Dinitrophenylhydrazine (DNPH) was conducted to determine whether any formaldehyde was present in the unidentified peak. In addition to the soil extract test sample, water served as a negative control, and water fortified with formaldehyde served as a positive control for the DNPH reaction. Analysis was by LC/TOF-MS with additional radioactive detection and using a third HPLC system which utilised a Luna C8(2) column (50  $\times$  2.0 mm, 3  $\mu\text{m}$ ).

A two-step derivatisation was performed to determine whether the polar metabolite was methanol. Extract 4 was subjected to enzymatic oxidation of any alcohols present with alcohol oxidase to their respective aldehydes, followed by reaction with 2,4-dinitrophenylhydrazine (DNPH) to generate 2,4-dinitrophenylhydrazones. Water

served as a negative control and water fortified with methanol and water fortified with formaldehyde served as the positive controls. Analysis was by reverse-phase LC/TOF-MS and reverse-phase HPLC with UV detection.

Ion exclusion chromatography on a Bio-Rad Aminex HPX-87H column, 300 x 7.8 mm, equipped with a refractive index detector, was performed on Extract 4. Additional radioactive flow detection was performed for selected analyses.

## II. RESULTS AND DISCUSSION

LC/TOF-MS spectra (retention times and mass) demonstrated that the peak in the soil extracts was not UDMH, dimethylamine or NDMA.

Following derivatisation with DNPH, comparison to chromatograms of the water + formaldehyde positive control demonstrated that trace amounts of formaldehyde were present in the soil extracts when analysed by LC/TOF-MS (Time Of Flight – MS). However, the amounts observed did not correspond to more than a small fraction of the total concentration of the peak, and when analysed with a radioactive detector, no response was obtained, indicating that the unknown metabolite had not been derivatised. Consequently, it was concluded that the trace amounts of formaldehyde observed in LC/TOF-MS corresponded to natural background concentrations of formaldehyde in soil and not to the unidentified metabolite.

In the two-step derivatisation process, formation of formaldehyde-DNPH was observed in the UV chromatograms of the positive control and in the UV-chromatograms for the extract. However large amounts of the 2,4-DNPH reagent remained unreacted and the yield was low, and a corresponding shift in the retention time of the unknown radio-chromatographic peak in the extract was not observed. The higher abundance of formaldehyde-DNPH in the soil extract appeared to have been due to the presence of background formaldehyde in the soil matrix which was consistent with the low yield observed for the methanol positive control. As this reaction scheme did not generate a high yield of formaldehyde-DNPH from the methanol positive control, the results from these experiments were considered inconclusive.

In ion exclusion HPLC with LC-Refractive Index (RI) detection the unknown radioactive peak was retained and was identified as methanol by comparison to standards for the candidate compounds, co-chromatography with methanol fortified extracts and refractive index and radioactivity flow detection in series. Formaldehyde and formic acid were excluded on the basis of comparison to reference standards.

## III. CONCLUSION

The results show that the unknown radioactive soil metabolite (M1) did not correspond to the reference substances for daminozide, UDMH, dimethylamine, NDMA, formic acid or formaldehyde. Analysis of the soil extracts by ion exclusion HPLC with refractive index detection, combined with radioactivity flow detection, and co-chromatography with reference standards, identified the metabolite as methanol.



**RMS comments and conclusion**

One soil extract was analysed only. ~~It is highly uncertain that unknown metabolite was methanol in all cases and in all soil extracts.~~ Because the work performed with LC/TOF-MS demonstrated that the metabolite was the same in all extracts, it is accepted to use the extract which gave the highest concentration of the metabolite only.

Identification of unknown peak by ion exclusion HPLC with LC-Refractive Index (RI) detection is based on comparison to standards and co-chromatography with methanol fortified extracts. The RMS is of opinion that it is likely that unknown peak is methanol; however, analysis of unknown metabolite should be confirmed by other specific method. The most appropriate method for methanol is gas-chromatography, NMR spectroscopy or Raman spectroscopy.

**B.8.1.1.1.2 Anaerobic degradation**

Degradation of daminozide under anaerobic conditions is presented in the study by Dzialo and Harned (1986), presented above (report no. A.8.1.5). Short summary is provided below.

A laboratory soil metabolism study with methyl-<sup>14</sup>C-daminozide was performed under dark aerobic conditions at 25°C for up to 16 hours. After 16 hours, anaerobic conditions were established by flooding with distilled water. Samples were taken immediately after flooding and at 3 other time-points; at 15 days, 30 days and at study termination after 60 days. Total recovery dropped from 103% AR immediately after application to 82.4% AR at t=0 (immediately after flooding) to 42.2% AR after 60 days. The low recoveries were stated to be due to the possible formation of methane and ethane, and due to the loss of CO<sub>2</sub> during the analysis procedure. Radioactivity in the aqueous filtrate and soil extractable radioactivity totalled 44.8% AR immediately after flooding, and declined rapidly to 4.7% AR after 15 days. Bound residues and CO<sub>2</sub> amounted to maxima of 25% AR and 20% AR after 15–30 days. Daminozide concentrations were 36.6% AR immediately after flooding and declined to 0.9% AR after 15 days. The maximum formaldehyde concentration was 5% AR after flooding at t=30 days. All other metabolites were < 5% AR.

The data do not allow a robust degradation rate calculation in accordance with FOCUS kinetics guidance. However, as a worst case the half-life of daminozide under anaerobic conditions was estimated as 7.5 days in the study report, based on the concentrations of daminozide at two data points (day 0 and day 15). This value was normalised to 20°C by the RMS using a Q<sub>10</sub> of 2.2, and reported as 11 days in the original DAR evaluation. Considering the revised recommended Q<sub>10</sub> value of 2.58, a temperature normalised anaerobic DT<sub>50</sub> value of 11.8 days is calculated.

**RMS comments and conclusion:**

Study has already been discussed above and it was concluded that it is not considered acceptable. DT<sub>50</sub> based on two data points is not accepted as reliable endpoint.

Statement provided by the notifier is presented below:

*It is not envisaged that anaerobic soil conditions will be encountered for the proposed ornamental glasshouse uses, while for the proposed outdoor uses, also to ornamentals, it is anticipated that application will not occur when the soil is under anaerobic conditions, and that the aerobic degradation rate of daminozide is so rapid that anaerobic conditions will never be encountered once daminozide is applied. Therefore, because of the lack of exposure of daminozide to anaerobic conditions from the proposed uses, and since the information in Dzialo and Harned (1986) indicates that anaerobic conditions do not liberate novel metabolites, no further data, beyond those previously assessed, are considered necessary.*

The RMS agrees that anaerobic conditions for the intended uses are unlikely to occur.

#### B.8.1.1.1.3 Soil photolysis

<b>Reference:</b>	<b>Lengen, M., Dzialo, D. (1984)</b> Daminozide Photolysis in Water and on Soil (Interim Report)
Report No.:	84246
Document No:	EPA guidelines
Guideline:	None
GLP:	No
<b>Previous evaluation:</b>	<b>In DAR (1999)</b>

#### Test item:

Test material: <sup>14</sup>C-labelled daminozide

Specific radioactivity: 5.682 mCi/mmol

Radiochemical purity: 98.5% (HPLC)

Duplicate 10 ml solutions with nominal concentrations of 200 ppm [<sup>14</sup>C]-daminozide in autoclaved quartz tubes (Southern New England Ultraviolet Company) were placed in a custom built photoreactor equipped with 2 x 3000A° and 2 x 3500A° lamps (Payonet, this combination of sunlight and black light is referred to as ultraviolet in the report, <290 nm). Light intensity was 1.36 watts/m<sup>2</sup> as measured with a YSI Kettering light meter. Samples were exposed to 30 equivalent 12-hour days of light and aliquots removed at intervals for quantitation of radioactivity and characterization of components in solution. An additional set of control samples were similarly prepared and kept in darkness. Under aseptic conditions, aliquots were removed for quantitation of radioactivity and HPLC analysis.

Soil samples of 5 g each (for soil analysis see table below) were treated with <sup>14</sup>C-daminozide to yield a final concentration of 75 ppm and exposed to ultraviolet light as above. The same number of control samples were prepared and incubated in darkness.

**Table B.8.1.1.1.3-1: Soil analysis**

Texture	Sandy loam
% Sand	67.6
% Silt	26.0
% Clay	6.4
pH	5.6
Organic matter (%)	7.3
Moisture retention at 1/3 Bar (%)	26.9
Cation exchange capacity (meq/100 g)	43.5

At intervals, samples were removed and stored frozen (0°C) until analyzed. The soils were extracted with 1% formic acid for 1 hour on a wrist action shaker, and centrifuged for 15 minutes at 4000 rpm. The supernatant was analyzed for radioactivity and components in solution characterized by HPLC.

#### Aqueous photolysis

Throughout the study, recovery of radioactivity was greater than 96%, indicating no volatile losses of photolytic product(s) occurred. Daminozide concentration remained stable in control samples, but a significant decrease was seen in irradiated solutions (Table B.8.1.1.1.3-2). The photolysis rate constant (k) was calculated as 0.00658/day, and half-life corresponded to 105 days. In control samples, a single radioactive peak (other than daminozide, 29 min) eluted at the retention time of 1,1-dimethylhydrazine and dimethylamine (33.5 min). This peak was also present in irradiated solutions, and increased slightly throughout the study.

**Table B.8.1.1.1.3-2: Concentration of daminozide in photolysis samples**

	ppm	
Time (days)	control	ultraviolet
<u>Aqueous</u> 0	193.1 <sup>a</sup>	202.8
4	216.6	163.6
10	213.3	173.3
18	198.8	172.2
24	217.4	181.1 <sup>a</sup>
30	213.8	153.1
k photolysis	-	0.00658
Half-life (days)	stable	105.3
Correlation coefficient		-0.76
<u>Soils</u> 0	48.0	49.0
4	40.2	43.2
10	42.7	37.1
18	43.9	38.3
24	32.5	29.6
30	29.3	30.2
k photolysis	0.01383	0.01588
Half-life (days)	50.1	43.6

Correlation coefficient	0.85	0.94
-------------------------	------	------

<sup>a</sup> data point omitted from linear regression analyses

**Table B.8.1.1.1.3-3: Concentration of daminozide products detected by HPLC analysis**

Time (days)	ppm UDMH/DMA <sup>a</sup>		ppm 4.5 min <sup>b</sup>		ppm cyclic daminozide (17 min) <sup>c</sup>	
	33 min					
Aqueous	Control	UV	Control	UV	Control	UV
0	0.6	0.8	0	0	0	0
4	0.2	0.3	0	2.1	0	0
10	0.3	0.4	0	4.5	0	0
18	0.2	0.5	0	6.9	0	0.2
24	0	0.7	0	9.9	0	0.3
30	0	0.6	0	9.9	0	0.3
Soils						
0	0.6	0.8	7.2	7.7	0	0.1
4	0.5	0.7	5.9	10.0	0	0.1
10	0.6	0.5	4.6	4.7	0.1	0.1
18	0.4	0.5	4.8	4.0	0.1	0.1
24	0.8	0.7	12.7	10.1	0.1	0.2
30	0.9	0.4	12.2	3.6	0	0.2

<sup>a</sup> calculated as ppm UDMH

<sup>b</sup> calculated as ppm daminozide

<sup>c</sup> calculated as ppm cyclic daminozide

#### Photolysis on soil

As summarized in Table B.8.1.1.1.3-4, an overall loss of radioactivity occurred in control soil, and this loss is slightly accelerated in irradiated soil. Levels of unextractable <sup>14</sup>C increase in both samples. Metabolism studies of daminozide in aerobic soil have established the rapid formation of CO<sub>2</sub> (Dannals, et al., 1972); the decrease in radiocarbon balance is likely to be due to this CO<sub>2</sub> formation, while the increase in unextractable material may indicate incorporation of this molecule into the soil matrix.

From HPLC analyses of soil extracts, daminozide concentration decreased in both samples. The half-life was calculated as 50.1 days and 43.6 days for control and photolyzed soils. This finding indicates that there is no significant photolytic loss of daminozide on soil.

As in aqueous photolysis samples, a major peak of radioactivity eluted at 4.5 min, and was tentatively characterized as formaldehyde. Radioactivity eluting at the retention time of 1,1-dimethylhydrazine/ dimethylamine was detectable in all soil extracts, and slightly increasing levels were detected in the controls (Table B.8.1.1.1.3-4). Levels of radioactivity chromatographing at 17 min, retention time of cyclic daminozide, did not exceed 0.1 ppm (Table B.8.1.1.1.3-5). Two other minor peaks at 30 minutes (N-formyldimethylhydrazone) and 37 minutes (unknown) were also detected, but no general trends were noted.

Table B.8.1.1.1.3-4: Recovery of radioactivity in soil photolysis samples

Time (days)	Extracted %	Unextracted %	Total %
Control			
0	72.8	18.1	90.9
4	58.5	15.8	74.3
10	62.1	22.7	84.8
18	58.2	20.6	78.8
24	60.8	28.0	88.8
30	57.9	26.1	84.0
Ultraviolet			
0	72.0	19.2	91.2
4	69.3	23.1	92.4
10	52.8	21.1	73.9
18	52.6	20.6	73.2
24	55.4	25.8	81.2
30	57.7	14.7	72.4

**RMS comments and conclusion**

Study was not conducted according to current guidelines. UV light of <290 nm was used that is not relevant for phototransformation of chemicals in the environment. Study is therefore considered not acceptable.

The notifier's statement to soil photolysis:

Spectra summarised in the original DAR assessment at B.2.1.10 indicate that daminozide does not absorb light above >290 nm, which indicates that soil photolysis is not expected to occur under natural conditions. Additional UV-vis absorption spectra from the study of Kelly (2011), which is summarised in dRAR, Volume 3, B-2, displayed negligible absorption of light at wavelengths >290nm. Therefore, daminozide would not be expected to undergo photolytic degradation, and additional data are not required.

The RMS agrees with the statement; soil photolysis study is not required.

<b>Reference:</b>	<b>Lengen, M. (1985)</b> Addendum to: Hydrolysis of daminozide (Project No. 84244) and Daminozide photolysis in water and on soil – Interim Report (Project No. 84246). Detection and Quantification of hydrazine and monomethylhydrazine, unsymmetrical Dimethylhydrazine, Dimethylamine and N-Nitrosodimethylamine Analyses in Daminozide Hydrolysis and Photolysis samples.
Report No.:	84244/84246 addendum
Document No:	-
Guideline:	None
GLP:	No
<b>Previous evaluation:</b>	In DAR (1999)

The analyses of parent compound and degradation products in daminozide hydrolysis and photolysis studies previously conducted (Proj. No. 84244 and 84246, Dec 1984) were carried out to quantitate UDMH, hydrazine and N-nitrosodimethylamine residues present in those studies. UD levels were quantified by a GC method and were found to be comparable to those previously obtained by HPLC. The presence of dimethylamine was not substantiated. No hydrazine was detected above a detection limit of 0.015 ppm, although 0.05 ppm of monomethylhydrazine was detected in a soil photolysis sample. No N-nitrosodimethylamine was found at a detection limit of 10 ppb using TEA analysis.

**Table B.8.1.1.3-5: Comparison of UDMH concentrations in daminozide samples analysed by HPLC and GC methods**

Sample	ppm UDMH/Dimethylamine (by HPLC)	ppm UDMH by derivatization/GC
Hydrolysis		
pH 1-30 day	26.3	33.5
pH 5-30 day	4.4	6.3
pH 7-30 day	0.7	0.04
pH 9-30 day	0.6	0.06
Photolysis		
Aqueous -		
control – 30 day	0.6	0.05
irradiated – 30 day	0	0.05
Soil -		
control – 0 day	0.6	1.43
control – 18 day	0.4	1.12
irradiated – 18 day	0.5	0.63
control – 30 day	0.9	5.61
irradiated – 30 day	0.4	4.82

#### RMS comments and conclusion

Study of photolysis (see above) and hydrolysis study (discussed under point B.8.2.1.1) are not considered acceptable; therefore, the addendum is not useful.

**B.8.1.1.2 Rate of degradation in soil****B.8.1.1.2.1 Laboratory studies, aerobic degradation of the active substance**

The study of Möndel, 2015, is summarised in section 8.1.1.1.1 above and is considered to provide the definitive end-points for the rate of degradation of daminozide in laboratory aerobic soil degradation studies. SFO DT<sub>50</sub> values of 0.1 – 0.4 days were calculated in all four soils for the aerobic degradation of daminozide in the dark at 20°C and 40% MWHC, for use in modelling. In two soils FOMC kinetics were considered most appropriate to derive end-points for comparison to persistence triggers by the notifier. The RMS is of opinion that SFO gave better visual fit and is acceptable also for persistence trigger endpoints. A summary of the degradation rates in the individual soils and correction to a soil moisture content of pF2 are presented in Table below. A geometric mean DT<sub>50</sub> for daminozide, corrected to 20°C and pF2 for use in modelling, of 0.12 days was calculated.

**Table B.8.1.1.2.1-1: Summary of the calculated DT<sub>50</sub> values for daminozide in aerobic soil degradation studies**

Soil, USDA classification	Kinetic model	pH (0.01M CaCl <sub>2</sub> )	Temp. (°C)	Soil Moisture (g/100g)	Water holding capacity at pF2 (g/100g)	Correction Factor	DT <sub>50</sub> /DT <sub>90</sub> (days)	χ <sup>2</sup> error (%)	Corrected DT <sub>50</sub> – 20°C & pF2 (days)
LUFA 2.4 – Loam	SFO	7.2	20	17.5	34.5	0.62	0.37/ 1.21	3.0	0.23
LUFA 2.2 – Loamy sand	SFO	5.5	20	17.0	14.0*	-	0.11/ 0.35	7.7	0.11
LUFA 5M – Sandy loam	SFO	7.3	20	15.7	19.0*	0.87	0.14/ 0.47	6.0	0.12
Fislis – Silt loam	SFO	6.8	20	12.8*	42.3	0.43	0.15/ 0.50	8.0	0.06
<b>Geometric Mean</b>									<b>0.12</b>

\* Standard values used from FOCUS (2012): Generic guidance for Tier 1 FOCUS groundwater assessments; V.2.1, Dec., 2012.

**B.8.1.1.2.2 Aerobic degradation of metabolites, breakdown and reaction products**

The polar metabolite fraction, M1, subsequently identified as methanol, was observed in the aerobic soil degradation study of Möndel, 2015, at maximum concentrations of 18.6 – 27.2% AR, 1-2 days after application, in all four soils. Reliable SFO degradation rates for methanol were calculated in accordance with FOCUS Kinetics guidance. A summary of the calculated aerobic degradation rates of methanol in the individual soils incubated at 20°C, and following correction to a soil moisture content of pF2 is presented in Table below. A geometric mean DT<sub>50</sub> corrected to 20°C and pF2 for use in modelling, of 3.9 days was calculated. A mean formation fraction of 0.27 was also calculated.

The List of End Points (LoEP) in the Review Report for daminozide reports that the metabolite formaldehyde was observed at a maximum concentration of 21% in one aerobic soil degradation study (Yu and Kobryn, 1993). The identification of the polar metabolite as formaldehyde in the original review is unreliable, and the polar metabolite observed in this study is most likely to be methanol. Though it is clear that the polar metabolite observed in the study of Yu and Kobryn, 1993, degrades rapidly in aerobic soil (concentrations decrease from a maximum concentration of 21% AR / 0.36 mg/kg after 16 hours to 1.2% AR / 0.02 mg/kg after 72 hours) degradation rates were not previously reported, and the data are not sufficient to allow a kinetic evaluation in accordance with FOCUS Kinetics guidance. Nevertheless, considering that over 90% degradation of the metabolite has occurred during the two time-points, it is possible to estimate an absolute worst case DT<sub>50</sub> of 56 hours, or 2.3 days, for the aerobic soil degradation of the metabolite in the existing study at 25°C on the basis of these two data-points.

The similarity of the degradation rates of methanol to the worst case DT<sub>50</sub> value calculated for the metabolite in the existing study of Yu and Kobryn, 1993, supports the conclusion that the metabolite is most likely the same compound, and therefore that it corresponds to methanol. Since the DT<sub>50</sub> values calculated from the existing studies are concluded to be unreliable, only degradation values from the study of Möndel, 2015 are considered in the exposure assessments.

**Table 8.1.1.2.2-1: Summary of the calculated DT<sub>50</sub> values for the metabolite M1 (methanol) following application of daminozide in aerobic soil degradation studies**

Soil, USDA classification	pH (0.01M CaCl <sub>2</sub> )	Temp. (°C)	Soil Moisture (g/100g)	Water holding capacity at pF2 (g/100g)	Correction Factor	DT <sub>50</sub> /DT <sub>90</sub> (days)	Formation Fraction	χ <sup>2</sup> error (%)	Corrected DT <sub>50</sub> – 20°C & pF2 (days)
LUFA 2.4 - Loam	7.2	20	17.5	34.5	0.62	6.2/20.5	0.25	24.6	3.8
LUFA 2.2 – Loamy sand	5.5	20	17.0	14.0*	-	6.1/20.1	0.29	18.9	6.1
LUFA 5M – Sandy loam	7.3	20	15.7	19.0*	0.87	5.9/19.4	0.26	18.3	5.1
Fisli – Silt loam	6.8	20	12.8*	42.3	0.43	4.5/15.0	0.29	18.3	1.9
<b>Geometric Mean</b>							-	-	<b>3.9</b>
<b>Arithmetic Mean</b>							<b>0.27</b>	-	<b>4.2</b>

\* Standard values used from FOCUS (2012): Generic guidance for Tier 1 FOCUS groundwater assessments; V.2.1, Dec., 2012.

## Field studies

See separate Annex B.8 for the product data.



**B.8.1.2 Adsorption and desorption in soil****B.8.1.2.1 Adsorption and desorption of the active substance**

<b>Reference:</b>	<b>Beeching, A. (1987)</b> Determination of Adsorption Isotherms for Daminozide on Two Soils
Report No.:	FAL 0018
Document No:	-
Guideline:	OECD 106
GLP:	No – Study performed prior to GLP being required
<b>Previous evaluation:</b>	In DAR (1999)

**Executive Summary:**

The adsorption and desorption of non-radiolabelled daminozide was studied in two soils. The study was conducted to OECD Guideline 106. The results of the study indicate low adsorption of daminozide in both soils. However, Freundlich isotherms could not be plotted, and  $K_{oc}$  values could not be calculated due to lack of information presented in the report.

**I. MATERIAL AND METHODS**

The adsorption and desorption of non-radiolabelled daminozide was studied in two soils, according to the OECD 106 guideline. The two soils were selected to represent a wide range of soil characteristics recommended in the guideline. Soil characteristics are presented in Table B.8.1.2.1-1. Prior to use soils were sieved through a 2 mm sieve.

Following application of the test item in 0.01 N calcium chloride solution, 10 g soils were equilibrated with an unstated volume of 0.01 N calcium chloride solution by shaking for between 1 and 15 hours, in a preliminary test. Five test concentrations were studied for the advanced adsorption isotherm test with concentrations of 0.5–5.0 mg/L. An unknown volume of aqueous solution was shaken with 10 g soil. The shaking duration for the advanced adsorption isotherm test was not reported. Following agitation the suspension was centrifuged, the volume of solution measured, and analysed for daminozide with HPLC equipped with a UV detector. A relative standard deviation (RSD) of 3.5% was reported for reference standards at 1.0 and 5.0 mg/L.

**Table B.8.1.2.1-1: Summary of soil characteristics**

	Soil I	Soil II
Particle size (% w/w):		
Clay	15	7
Silt	-	-
Sand	-	-
Texture (US)	Loam	Sand
pH (water)	7.5	5.1
Organic Matter (%)	1.4	3.3
Organic Carbon (%)	0.8	1.9
CEC (meq/100 g soil)	12.3	13.6

## II. RESULTS AND DISCUSSION

The concentration of daminozide remaining in solution in the advanced adsorption isotherm study is shown in Table B.8.1.2.1-2. The report's author stated that Freundlich isotherms could not be calculated because sorption was within the standard deviation of the analytical method. This is not correct for all samples, and Soil I displays a small amount of adsorption for the majority of test concentrations. For Soil II, the concentration in solution at equilibrium is consistently higher than the nominal stated initial concentration, indicating that adsorption in this soil is minimal and also suggesting that the initial test concentration was higher than nominal. However, it remains impossible to calculate Freundlich isotherms for either soil because neither an aqueous phase volume, nor measured soil concentrations at equilibrium are reported.

**Table B.8.1.2.1-2: Adsorption of daminozide in the advanced adsorption isotherm test**

Soil type	Initial concentration in aqueous phase (C <sub>i</sub> ) [µg/mL]	Concentration in aqueous phase at equilibrium (C <sub>e</sub> ) [µg/mL]
Soil I	0.5	0.49
	1.0	0.93
	2.0	1.79
	3.0	2.84
	5.0	5.06
Soil II	0.5	0.5
	1.0	1.15
	2.0	2.15
	3.0	3.15
	5.0	5.09

## III. CONCLUSION

The results of the study indicate low adsorption of daminozide in both soils. However, Freundlich isotherms could not be plotted, and K<sub>oc</sub> values could not be calculated due to lack of information presented in the report.

**RMS comments and conclusion**

Several deviations were found in the study report:

- Purity of daminozide was not specified.
- Soil/water ratio not reported.
- Aqueous phase volume and measured soil concentrations at equilibrium not reported
- No K<sub>oc</sub> values can be calculated

Study is therefore not considered acceptable.

<b>Reference:</b>	<b>Spare, W. (1987)</b> Determination of the Adsorption/Desorption Constants of ALAR
Report No.:	A.8.1.11
Document No:	-
Guideline:	US EPA guideline Subdivision N: Series 163-1
GLP:	Yes
<b>Previous evaluation:</b>	In DAR (1999)

Test compound: [<sup>14</sup>C-methyl]-daminozide

Specific activity: 21.7 mCi/mM

Radiochemical purity: 92.1%

**Executive Summary**

The adsorption and desorption of [<sup>14</sup>C-methyl]-daminozide was studied in four soils. The study was conducted according to US EPA guideline, Subdivision N: Series 163-1, and in accordance with GLP.

The soil and 0.01 N calcium ion solution was sterilised prior to the addition of daminozide. Analysis by LSC displayed radioactive mass balances in the preliminary test after 48 hours shaking of 98.2–102.4% AR. The results show that adsorption equilibrium had effectively been reached after 2 hours shaking, with only very minor changes in aqueous phase radioactivity being observed in later samples. It can be seen from the results presented for 2 – 48 hours, that shaking time does not significantly affect the results of the study over this time period.

Freundlich adsorption and desorption parameters were obtained for daminozide. Freundlich adsorption coefficients (K<sub>foc</sub>) were in the range 18.4 to 46.5 L/kg. The range of 1/n values was 1.107 to 1.368.

Desorption K<sub>foc</sub> values ranged from 612 L/kg to 47337 L/kg. However, the values reported may be affected by the low amounts of radioactivity remaining in soil following the adsorption phase of the study.

## I. MATERIAL AND METHODS

The adsorption and desorption of [ $^{14}\text{C}$ -methyl]-daminozide was studied in four soils. The soils were selected to provide an appropriate range of organic carbon contents, soil pH values, clay contents and soil classifications. Soil characteristics are presented in Table B.8.1.2.1-3. Prior to use soils were oven-dried at 90°C, sterilised by autoclaving, and sieved through a 2 mm sieve. Preliminary investigations were performed to determine the appropriate ratio of soil to solution, adsorption and desorption equilibrium times, and material balance. Duplicate samples of 1 g soil: 20 mL of aqueous 0.01 N calcium ion solution (prepared from sterilised deionised and distilled water) was shaken for between 2 and 48 hours. Following shaking all samples were centrifuged and analysed by LSC. The mass balance was determined in the preliminary study samples for each soil after 48 hours of shaking. The radioactivity in extracted soil was quantified by combustion with LSC.

For the main test of the definitive adsorption study, duplicate 5 g soil samples were dispensed into individual polypropylene test vessels. 25 mL of sterilised 0.01 M calcium ion solution containing [ $^{14}\text{C}$ ]-daminozide was added to the test vessels to give five test concentrations of 0.2-10  $\mu\text{g/mL}$  (0.2, 0.5, 1, 5, 10  $\mu\text{g/mL}$ ). Following application of the test item test vessels were continuously shaken in a temperature controlled room (25–26°C) for 6 hours. Following shaking all samples were centrifuged and analysed by LSC.

**Table B.8.1.2.1-3: Summary of soil characteristics**

	Maryland Clay	Maryland Sand	Mississippi	California
Particle size (% w/w):				
Clay	42.0	2.2	11.2	6.4
Silt	32.8	2.2	39.2	19.6
Sand	25.2	95.6	49.6	74.0
Texture (USDA)	Clay	Sand	Loam	Sandy Loam
pH*	5.9	6.5	7.6	6.5
Organic Matter (%)	4.8	0.9	1.2	0.5
Organic Carbon (%)	2.8	0.5	0.7	0.3
CEC (meq/100 g soil)	24.3	1.8	8.0	4.7
Moisture at Field capacity	35.9	3.8	13.3	6.1

\* medium in which the pH was measured is not stated

Following removal of the adsorption supernatant from the main test vessels, 25 mL of 0.01 N calcium ion solution was added, and the test vessels shaken for a further 6 hours. Test vessels were then centrifuged, and the radioactivity in the supernatant determined by LSC.

## II. RESULTS AND DISCUSSION

In the preliminary test mass balances after 48 hours were 98.2 – 102.4% AR. The amount of radioactivity remaining in solution in the adsorption equilibration time study is shown in Table B.8.1.2.1-4. The results show that adsorption equilibrium had effectively been reached after 2 hours shaking, with only very minor changes in aqueous phase radioactivity being observed in later samples. It can be seen from the results presented for 2 – 48 hours, that shaking time does not significantly affect the results of the study over this time period. On the basis of the preliminary test, a 6-hour shaking time and a soil: solution ratio of 1:5 was selected for the main test.

**Table B.8.1.2.1-4: Adsorption of daminozide during the Time to Adsorption Equilibrium Test**

Soil type	Time (Hours)	Concentration in aqueous phase (µg/mL)	Concentration on soil (µg/g)	K <sub>d</sub>	K <sub>oc</sub>
Maryland Clay	2	13.36	-	0.76	27.1
	4	13.51	-	0.76	27.1
	8	13.65	-	0.75	26.7
	24	13.47	-	0.76	27.1
	48	13.62	0.015	0.75	26.7
Maryland Sand	2	13.77	-	0.00	0.0
	4	13.86	-	0.00	0.0
	8	14.16	-	0.00	0.0
	24	13.94	-	0.00	0.0
	48	13.91	10.15	0.00	0.0
Mississippi Loam	2	14.07	-	0.18	25.7
	4	13.84	-	0.18	25.7
	8	14.09	-	0.18	25.7
	24	14.05	-	0.18	25.7
	48	13.79	2.47	0.18	25.7
Iowa Sandy Loam*	2	13.46	-	0.45	15.5
	4	13.35	-	0.45	15.5
	8	13.87	-	0.44	15.1
	24	13.64	-	0.45	15.5
	48	13.66	6.06	0.45	15.5

\* Iowa sandy loam (2.9% OC) was replaced by the California Sandy Loam in the main test

Concentrations of radioactivity in the main adsorption isotherm test supernatants and soil are shown in Table B.8.1.2.1-5 to Table B.8.1.2.1-8. The coefficients  $K_f$ ,  $K_{foc}$  and  $1/n$  values are presented in Table B.8.1.2.1-9 for the adsorption and desorption phases. Correlations were good for all adsorption and desorption phase Freundlich isotherms, indicating the good reliability of the results. Calculated adsorption  $K_{foc}$  values were 18.4 – 46.5 mL/g indicating low sorption. Desorption  $K_{foc}$  values were significantly higher (612 – 47337 mL/g) indicating significant hysteresis of adsorbed daminozide. However, reported desorption  $K_f$  and  $K_{foc}$  values may be affected by the small amounts of radioactivity remaining in soil following the adsorption phase of the test.

**Table B.8.1.2.1-5: Mean concentrations of daminozide (based on distribution of radioactivity) in Maryland Clay soil**

Test Concentration (µg/mL)	Adsorption		Desorption	
	C <sub>e</sub> (µg/mL)	X/m (µg/g)	C <sub>e</sub> (µg/mL)	X/m (µg/g)
0.20	0.177	0.105	0.0072	0.0715
0.47	0.434	0.204	0.0180	0.116
1.00	0.874	0.621	0.042	0.412
5.03	4.40	3.21	0.177	2.32
10.31	8.86	7.37	0.357	5.56

**Table B.8.1.2.1-6: Mean concentrations of daminozide (based on distribution of radioactivity) in Maryland Sand soil**

Test Concentration (µg/mL)	Adsorption		Desorption	
	C <sub>e</sub> (µg/mL)	X/m (µg/g)	C <sub>e</sub> (µg/mL)	X/m (µg/g)
0.20	0.197	0.010	0.00016	0.010
0.47	0.487	na	na	na
1.00	0.975	0.118	0.0014	0.115
5.03	0.492	0.567	0.008	0.527
10.31	9.89	2.08	0.01	2.05

na – no value available

**Table B.8.1.2.1-7: Mean concentrations of daminozide (based on distribution of radioactivity) in Mississippi Loam soil**

Test Concentration (µg/mL)	Adsorption		Desorption	
	C <sub>e</sub> (µg/mL)	X/m (µg/g)	C <sub>e</sub> (µg/mL)	X/m (µg/g)
0.20	0.193	0.033	0.0023	0.0206
0.47	0.475	0.010	0.0025	na
1.00	0.961	0.192	0.0051	0.167
5.03	4.81	1.105	0.029	0.963
10.31	9.66	3.28	0.068	2.94

na – no value available

**Table B.8.1.2.1-8: Mean concentrations of daminozide (based on distribution of radioactivity) in California Sandy Loam soil**

Test Concentration (µg/mL)	Adsorption		Desorption	
	C <sub>e</sub> (µg/mL)	X/m (µg/g)	C <sub>e</sub> (µg/mL)	X/m (µg/g)
0.20	0.193	0.035	-	-
0.47	0.474	0.012	-	-
1.00	0.958	0.209	-	-
5.03	4.82	1.05	-	-
10.31	9.68	3.06	-	-

- the results reported for the desorption phase of the California Sandy Loam contain clear errors which are not repeated here.

**Table B.8.1.2.1-9: Summary of K<sub>F</sub>, K<sub>FOC</sub> and 1/n values from the adsorption and desorption steps**

Soil	Freundlich Evaluation				
		K <sub>F</sub>	K <sub>FOC</sub>	1/n	r <sup>2</sup>
Maryland Clay	Adsorption	0.642	23.0	1.107	0.9941
	Desorption	17.0	612	1.160	0.9872
Maryland Sand	Adsorption	0.096	18.5	1.285	0.9896
	Desorption	247.1	47337	1.156	0.9534
Mississippi Loam	Adsorption	0.128	18.4	1.368	0.8582
	Desorption	123.9	17808	1.361	0.9614
California Sandy Loam	Adsorption	0.135	46.5	1.315	0.8627
	Desorption	-	-	-	-

- the results reported for the desorption phase of the California Sandy Loam contain clear errors which are not repeated here.

### III. CONCLUSION

The soil and 0.01 N calcium ion solution was sterilised prior to the addition of daminozide. Analysis by LSC displayed radioactive mass balances in the preliminary test after 48 hours shaking of 98.2–102.4% AR. The results

show that adsorption equilibrium had effectively been reached after 2 hours shaking, with only very minor changes in aqueous phase radioactivity being observed in samples up to 48 hours.

Freundlich adsorption and desorption parameters were obtained for daminozide. Freundlich adsorption coefficients ( $K_{\text{foc}}$ ) were in the range 18.4 to 46.5 L/kg. The range of  $1/n$  values was 1.107 to 1.368.

Desorption  $K_{\text{foc}}$  values ranged from 612 L/kg to 47337 L/kg. However, the values reported may be affected by the low amounts of radioactivity remaining in soil following the adsorption phase of the study.

**Table B.8.1.2.1-10: Adsorption  $K_{\text{f}}$ ,  $K_{\text{foc}}$  and  $1/n$  (Freundlich exponent) values for daminozide**

Soil Selection	Soil pH	$K_{\text{f}}$ [mL/g]	$K_{\text{foc}}$ [mL/g]	$1/n$
Maryland - Clay	5.9	0.642	23.0	1.107
Maryland – Sand	6.5	0.096	18.5	1.285
Mississippi - Loam	7.6	0.128	18.4	1.368
California- Sandy Loam	6.5	0.135	46.5	1.315
<b>Arithmetic mean</b>		<b>0.250</b>	<b>26.6</b>	<b>1.269</b>

#### **RMS comments and conclusion**

Study was performed according to US EPA guideline Subdivision N: Series 163-1. A review of the study indicates that it partly meets the OECD 106 test guideline. The main deviations include:

Radiochemical purity of the test compound was 92.1% only. Soils were oven-dried at 90°C and sterilised by autoclaving. Three out of 4 soils had low organic carbon content (0.3 – 0.7%). For the chemical that are slightly adsorbed, a soil/solution ration of 1:1 is recommended. Results are not accurate if  $K_{\text{d}} \times \text{soil/water ratio} < 0.1$  (decline in concentration too small). Results with  $1/n > 1.1$  show concentration dependent sorption behaviour.  $K_{\text{oc}}$  for the three soils (18, 19, and 47 l/kg) are therefore not accurate and are concentration dependent.

In the original DAR it is stated that because of the  $\text{pK}_{\text{a}}$  of daminozide of 4.68, only results from soil with  $\text{pH} > 6.5$  are accurate. The result for the clay ( $K_{\text{oc}}$  23 l/kg) might be overrated due to ionisation of daminozide. The Notifier's statement to that is presented below.

In conclusion, the study is not considered valid.

#### **Notifier's statement:**

*In the original DAR it is stated that because the  $\text{pK}_{\text{a}}$  of daminozide is 4.68, only results from soils of  $\text{pH} > 6.5$  describe the behaviour of the anion accurately. However, the range of soils studied is from  $\text{pH}$  5.1–7.6, and no  $\text{pH}$  dependence is observed. Even if the two soils from the study of Beeching (1987), for which accurate  $K_{\text{foc}}$  values could not be determined, are excluded, no  $\text{pH}$  dependence is observed from the remaining four soils with  $\text{pH}$  values*

of 5.9–7.6. This is as would be predicted by the Henderson-Hasselbalch equation from which pH may be calculated from a given pKa:

$$pH = pK_a + \log_{10}([A^-]/[HA])$$

where:  $[HA]$  = molar concentration of undissociated weak acid

$[A^-]$  = molar concentration of the dissociated anion

Thus it can be calculated that at pH 5.1, 72% of daminozide would be present in its dissociated form<sup>1</sup>, at pH 5.6, 90% would exist in its dissociated form, and at pH 6.0, 95%. Therefore, for the soils studied the dominant form from pH 5.1, and especially from pH 5.6, is dissociated daminozide, and any changes in its concentration are minor. Thus, it is entirely expected that in this case soil pH would have no observable effect on the calculated  $K_{oc}$ . All soils are considered to be appropriate for use in risk assessment and for the calculation of a mean  $K_{oc}$  value for use in modelling.

~~RMS: It is not clear how results of 0.72 for  $[A^-]$  and 0.28 for  $[HA]$  from the ratio of 2.63 was achieved.~~ Study had several deviations and results are questionable.  $K_{oc}$  of 23 should be excluded due to pH below 6.5. The RMS proposed to use worst case  $K_{oc}$  of 18.4 ml/g and corresponding 1/n value of 1.368 for the risk assessment. New adsorption/desorption study has been performed by the Notifier and is requested.

#### B.8.1.2.2 Adsorption and desorption of metabolites, breakdown and reaction products

The polar metabolite M1, which was subsequently identified as methanol, was observed at concentrations which trigger identification and risk assessment in soil aerobic degradation studies. However, it was not possible to perform reliable batch adsorption studies for methanol because of the practical difficulties created by its high volatility (vapour pressure  $1.69 \times 10^4$  Pa at 25°C; Henry's Law Constant  $0.46 \text{ Pa}\cdot\text{m}^3\cdot\text{mol}^{-1}$  at 25°C (both values from EPIWEB 4.1 experimental database)), difficulties in its analysis and natural background concentrations in soil and water. Instead QSAR calculations were performed using the EPIWEB 4.1 software tool, and specifically the KOCWIN v 2.0 tool.  $K_{oc}$  values of 1.0 L/kg using the MCI method, 1.224 L/kg using the Log $K_{ow}$  method and 2.75 L/kg from the experimental database were obtained.

For comparative purposes, the  $K_{oc}$  of daminozide was also calculated using the same software tool. An estimated  $K_{oc}$  of 10 L/kg from the MCI method and 0.19 L/kg from the Log $K_{ow}$  method were obtained. The values (particularly that derived by the MCI method) are consistent with the experimental values reported above for daminozide demonstrating the reliability of the model calculations.

No further batch adsorption data are therefore necessary.

#### RMS comments and conclusion:

$K_{oc}$  value of 2.75 L/kg obtained from the experimental database is very uncertain as no details about the experiment are presented. QSAR calculation of  $K_{oc}$  is not recommended for use in GW models and FOCUS SW

<sup>1</sup> Using the Henderson-Hasselbalch equation at pH 5.1 with a pKa of 4.68:  $5.1 = 4.68 + \log_{10}([A^-]/[HA]) \rightarrow 0.42 = \log_{10}([A^-]/[HA]) \rightarrow 2.63 = [A^-]/[HA]$  which, by iteration, results in  $[A^-] = 0.72$ ;  $[HA] = 0.28$ .



Step 3 but in this case the worst case value of 1.0 L/kg is proposed for the risk assessment. Worst case value is considered conservative and is accepted by the RMS.

#### **B.8.1.2.3 Aged sorption**

No data were evaluated during the first EU review, and none were required. In accordance with Comm. Reg. (EU) No. 283/2013, which sets out the active substance data requirements in accordance with Comm. Reg (EC) No. 1107/2009, no additional data are submitted and none are required.

#### **B.8.1.3 Mobility in soil**

##### **B.8.1.3.1 Column leaching studies**

##### **B.8.1.3.1.1 Column leaching of the active substance**

<b>Reference:</b>	<b>McManus, J., Dyialo, D., Lengen, M. (1984)</b> Daminozide Column Leaching Study with Aged Sandy Loam
Report No.:	A.8.1.8
Document No:	-
Guideline:	None stated
GLP:	No – Study performed prior to GLP being required
<b>Previous evaluation:</b>	In DAR (1999)

Test compound: methyl labelled daminozide

Radioactive purity: 98.5%

Specific activity: 5.94 mCi/mmol

An aged leaching study with daminozide was performed. Daminozide was labelled in the methyl position.

Aging experiment: Two flasks containing 50 g of wet soil were treated with 18.4 µL daminozide (7.6 kg daminozide/ha). Each flask contained a trapping tower to monitor the release of <sup>14</sup>C-volatiles and carbon dioxide. Volatiles were trapped on foam plugs whereas <sup>14</sup>CO<sub>2</sub> was collected on ascarite. The soils were incubated for 2 days at 25 °C. One soil was extracted two times for 30 min with 150 ml 1% formic acid only after 2 days. The other was used for the column experiment.

Leaching experiment: The treated soil from one flask, two days after chemical application was transferred to the top of the soil column. Characteristics of column: I.D 4.9 cm, 30.5 cm untreated sandy loam and c. 6 cm treated soil. The soil was adjusted to field capacity (26.9%). Daminozide was eluted from the column with distilled water, which was collected in ten 100 mL segments. One mL of each eluant fraction counted directly by LSC. The soil column was dismantled into four 7.6 cm segments. Extraction as mentioned above. Detec. method: LSC, HPLC, combustion, and GC. Recovery of combustion: 96%.

**Table B.8.1.3.1.1-1: Analysis of soil used in leaching column**

	Oxford sandy loam
pH	5.6
% organic matter	7.34
Bulk density (gm/mL) (as received)	1.1963
Bulk density (gm/mL) (air dry)	1.2334
% Moisture retention at 1/3 bar	26.88
Cation exchange capacity (CEC) (meq/100 gm)	43.5
Texture:	Sandy loam
% sand	67.6
% silt	26.0
% clay	6.4

## Results

*Ageing study:* Seventy percent of r.a. was extractable. Bound residue amounted to 21%. Analysis of the soil extract by HPLC showed three peaks in the chromatogram. Analysis showed daminozide to be present at a concentration of 28.8% AR, high formation of a polar fraction (36.4% AR) which it was postulated in the DAR, could be formaldehyde, as well as a non-polar fraction at concentrations <5% AR. The less polar component had the same retention time as dimethylamine. These metabolites are normally rapidly further degraded in soil mainly to CO<sub>2</sub>, however, no volatiles were observed over the 2 days of ageing.

*Leaching study:* Total recovery of radioactivity applied to the soil column was 98%, this included 56% in the leachate, 39% bound (66% in the first 7.6 cm) and 3% extractable. Analysis of the radioactivity extracted from the soil sections showed polar products when analysed by HPLC. A total of 56.3% AR was present in the first 400 mL of leachate. The two fractions containing the majority (84.3%) of the leached radioactivity (equivalent to 47.5% AR) were analysed; only daminozide was detected in these fractions. All remaining fractions in the leachate comprised < 5% AR.

Analysis of the <sup>14</sup>C extracted from the soil showed only polar products.

## RMS comments and conclusion:

No information on time of leaching. Study was not conducted according to any guideline, not GLP and is considered as supplemental.

### B.8.1.3.1.2 Column leaching of metabolites, breakdown and reaction products

An aged residue column leaching study was performed with daminozide. Following 2 days of ageing two metabolite fractions were observed in soil. However, neither fraction was observed to leach through the soil column. See B.8.1.3.1.1 for further details.

**B.8.1.3.2 Lysimeter studies**

No lysimeter studies were evaluated during the first EU review, and none were required. In accordance with Comm. Reg. (EU) No. 283/2013 which sets out the active substance data requirements in accordance with Comm. Reg (EC) No. 1107/2009, lysimeter studies are only required where necessary. Data to assess the leaching of daminozide and its soil metabolites are presented above. Therefore, further data are not considered necessary here.

**B.8.1.3.3 Field leaching studies**

No field leaching studies were evaluated during the first EU review, and none were required. In accordance with Comm. Reg. (EU) No. 283/2013 which sets out the active substance data requirements in accordance with Comm. Reg (EC) No. 1107/2009, field leaching studies are only required where necessary. Adequate data to assess the leaching of Daminozide and its soil metabolites are presented above. Therefore, further data are not considered necessary here.

**B.8.2 Fate and behaviour in water and sediment****B.8.2.1 Route and rate of degradation in aquatic systems (chemical and photochemical degradation)****B.8.2.1.1 Hydrolytic degradation**

<b>Reference:</b>	<b>Lengen, M. (1982)</b> Hydrolysis of Daminozide
Report No.:	A.8.1.12
Document No:	-
Guideline:	-
GLP:	No
<b>Previous evaluation:</b>	In DAR (1999)

Test compound: [unsymmetrical dimethylhydrazine-<sup>14</sup>C] daminozide

Specific activity: 1.3 or 0.5 mCi/mM

Radiochemical purity: 95.1% and 86.1% respectively by TLC/LSC

The hydrolysis of 100 ppm daminozide as [unsymmetrical dimethylhydrazine- $^{14}\text{C}$ ]daminozide in buffered aqueous solutions of pH 5, 7 and 9 were heated to 25°C, 50°C and 70°C and adjusted with 0.1N HCl or 0.1N NaOH to the respective pH. Solutions were incubated in darkness at 25°C, 50°C and 70°C for up to thirty days and at various intervals were analyzed for radioactivity.

Recovery of radioactivity generally exceeded 95%.

Aqueous solutions of daminozide incubated at 25°C showed no apparent hydrolysis of the parent molecule. As indicated in Table B.8.2.1.1-1, no decrease in the amount of daminozide at pH 5, 7 or 9 was noted, as determined by thin-layer chromatography (TLC)/liquid scintillation counting (LSC).

In buffered solution at pH 5 at higher temperatures, a significant decrease in daminozide concentration was noted (Table B.8.2.1.1-1). Half-life of the parent compound was calculated to be 77.0 days and 6.9 days at 50°C and 70°C respectively. Extrapolation of the hydrolysis rate constant ( $k$ ) to 22°C yielded  $1.7 \times 10^{-4} \text{ day}^{-1}$ , with a corresponding half-life of daminozide of 4077 days. At pH 5, daminozide hydrolyzed to form unsymmetrical dimethylhydrazine and unidentified polar material. At 70°C, unsymmetrical dimethylhydrazine (UDMH) concentrations ranged from 7 to 28 µg/ml (Table B.8.2.1.1-1). Low levels ( $\leq 7$  µg/ml) of an unidentified polar material were produced at 50°C (Table B.8.2.1.1-2). However, when temperature was increased to 70°C, a concomitant increase in this product (23.5 µg/ml after 14 days) was observed.

In neutral solution (pH 7) daminozide was stable at lower temperature but hydrolyzed at 70°C with a rate constant of  $6.0 \times 10^{-3} \text{ day}^{-1}$  and a half-life calculated as 115.5 days (). Levels of UDMH ranged from 4.3 µg/ml to 10.0 µg/ml () and formation of polar material did not exceed 2.3 µg/ml.

In buffered solution at pH 9, daminozide was stable.

**Table B.8.2.1.1-1: Hydrolysis of daminozide at 25, 50 and 70°C. Concentration (µg/ml) vs. time (days)**

Time (days)	pH 5			pH 7			pH 9		
	25	50	70°C	25	50	70°C	25	50	70°C
0	87.1	95.5	81.9	81.2	84.1	80.4	88.2	91.1	89.3
1		90.7	66.1		83.6	80.6		90.1	91.6
2	81.4			76.1			89.6		
3		87.8	36.3		81.0	75.6		87.0	92.2
7	82.4	83.3	28.4	85.1	82.4	74.4	89.9	88.2	88.2
10	81.0	81.8	29.7	83.0	87.5	75.5	88.8	88.1	89.8
14	86.7	84.0	(46.1) <sup>a</sup>	89.7	85.9	74.2	91.4	89.5	91.1
18	90.2			92.6			90.0		
24	89.6			85.4			91.6		
30	95.0			95.9			97.6		
Half-life (days)	stable	77	6.9	stable	stable	115.5	stable	stable	stable

<sup>a</sup> Omitted from linear regression analysis

**Table B.8.2.1.1-2: Concentration of daminozide hydrolytic products vs. time**

Time (days)	µg UDMH/ml				µg polar material/ml	
	pH 5		pH 7		pH 5	
	50°C	70°C	50°C	70°C	50°C	70°C
0	nd	nd	nd	4.3	nd	nd
1	nd	7.2	4.2	7.7	0.3	11.3
3	nd	16.3	8.6	10.0	2.3	32.1
7	nd	28.5	5.3	9.4	6.2	21.7
10	nd	24.9	nd	7.2	7.1	24.8
14	nd	15.5	nd	8.6	4.9	23.5

- Not detectable

**RMS comments and conclusion:**

Study is considered acceptable. Daminozide is stable to hydrolysis at pH 5-7 at 25°C.

<b>Reference:</b>	<b>Lengen, M., Abdel-Kader, H., Peterson, G. (1984)</b> Hydrolysis of Daminozide
Report No.:	A.8.1.4
Project No:	84244
Guideline:	EPA registration standard (June 29, 1984)
GLP:	No
<b>Previous evaluation:</b>	In DAR (1999)

Test material: <sup>14</sup>C-methyl-label daminozide

Radiochemical purity: 98.5% (HPLC)

Specific activity: 5.682 mCi/mmol

Lot No.: #83-78-97

**Description**

A hydrolysis study with daminozide (98.5% pure, <sup>14</sup>C-methyl-label) was performed under sterile conditions in the dark. Test concentrations 200 mg/l nominal. Sampling after 0, 1, 5, 12, 20, and 30 days. Analysis by HPLC-r.a. (recovery >90%).

**Results**

Total recoveries >97%. Actual concentrations at t = 0 76-88% of nominal. At pH 5-9 concentrations increase after t = 0.

No hydrolysis at pH 5-9. At pH 1 DT50 amounts to 41 days (extrapolated value, r<sup>2</sup> 0.99).

UDMH (1,1-dimethylhydrazine) levels were: max. 26.3% at pH 1 and 4.4, 0.7%, and 0.6% at pH 5-9, all after 30 days. NDMA (1,1-dimethylnitrosamine), or dimethylamine (DMA) were observed, but could not be quantitated.

Hydrazine levels were never above the 15 µg/l level.

**Table B.8.2.1.1-3: Daminozide concentration (ppm) in aqueous buffered solutions incubated for thirty days**

	ppm			
Time (days)	pH1	pH5	pH7	pH9
0	175.2	174.3	153.1	151.6
1	177.0	183.2	193.1	187.0
5	174.9	186.7	191.9	185.9
12	144.1	184.9	195.1	167.2
20	131.6	179.8	195.2	195.1
30	107.1	185.3	199.3	186.1

**RMS comments and conclusion:**

Buffer types are not reported. At pH 5-9 concentrations daminozide increase after  $t = 0$ . DT50 at pH 1 is extrapolated. The result for pH 1 is not useful. The results are not used for risk evaluation.

**B.8.2.1.2 Direct photochemical degradation**

<b>Reference:</b>	<b>Brice, A., Scholey, A. (2006)</b> [ $^{14}\text{C}$ ]-Daminozide: Photodegradation in Sterile, Aqueous solution.
Report No.:	2242/044
Document No:	-
Guideline:	JMAFF Test Guidelines Section 2-6-2
GLP:	Yes
<b>Previous evaluation:</b>	Submitted for the purpose of renewal

Test compound: [1,4- $^{14}\text{C}$ ]-daminozide

Batch number: SEL/1678

Specific activity: 13.07 MBq/mg

Radiochemical purity: 99.4%

**Executive Summary**

The aqueous photolytic degradation of [1,4- $^{14}\text{C}$ ]-daminozide was studied in both pure (HPLC grade) water and in a natural stream water, at  $25 \pm 2^\circ\text{C}$ . The study was conducted to JMAFF Test Guidelines Section 2-6-2, and in accordance with the principles of GLP.

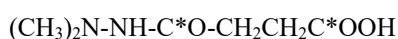
Sterilised waters were continuously irradiated with a xenon lamp, filtered to remove light  $<290\text{ nm}$ , for a maximum of 15 days. Dark control samples of both the natural and pure water were treated at the same rate and incubated.

Recoveries of individual samples were  $\geq 96.2\%$  AR in all cases. In all cases, degradation of daminozide was slow, with the majority of radioactivity being associated with parent daminozide in the aqueous phase. In the sterilised natural water 75% AR was unchanged daminozide at study termination, with 5.2% AR as an unknown metabolite (Unknown-2), and 18.5% AR as  $\text{CO}_2$ . In the irradiated pure water 94% AR was parent daminozide at the study

termination; Unknown-2 was not detected throughout the study duration, and concentrations of  $^{14}\text{CO}_2$  never exceeded 0.2% AR. Daminozide was essentially stable in the sterilised dark control samples.

## I. MATERIAL AND METHODS

The aqueous photolytic degradation of [1,4- $^{14}\text{C}$ ]-daminozide was studied in both pure (HPLC grade) water and in a natural stream water, at  $25 \pm 2^\circ\text{C}$ , over a 15 day period. The specific activity of the radio-labelled test item was 13.07 MBq/mg, with a radiochemical purity of 99.4%. The position of the radiolabels is as shown in Figure below.



**Figure B.8.2.1.2-1: Position of radiolabels for daminozide**

Natural water was obtained from the Chevin Forest Park stream and characterised. The characterisation of the natural and pure water is shown in TableB.8.2.1.2-2. Prior to use 25 mL of either natural or pure water was dispensed into individual autoclaved test vessels through a 0.2  $\mu\text{m}$  sterile filter. Waters were treated at a nominal application rate of 2.5 mg [ $^{14}\text{C}$ ]-daminozide/L, and the samples were continuously irradiated with a xenon lamp filtered to remove light  $<290\text{ nm}$  for a maximum of 15 days. Irradiated samples were sealed with a polyurethane bung, and connected to a series of traps (ethanediol, 2% paraffin in xylene, and 2 M NaOH), to allow any volatile degradation products to be retained. Single samples were collected immediately after application, at study termination, and at five intermediate sample times.

Dark control samples of both the natural and pure water were treated at the same rate and incubated. A single sample was analysed 15 days after treatment.

**TableB.8.2.1.2-2: Characteristics of natural and pure water samples**

	Natural Water	Natural water (filtered)	Pure water
Suspended solids (mg/L)	22	2	-
oxygen content (%)	98	96	-
Conductivity ( $\mu\text{S}$ )	410	366	28
pH	7.5	7.3	7.3

The radioactivity in samples and trapping solutions was quantified by LSC (the limit of detection for LSC was *ca*  $<0.1\%$  of applied radioactivity), and that in the aqueous phase characterised by HPLC (the limit of detection for LSC was *ca* 0.1% of applied radioactivity) equipped with both a radioactivity and UV detector, and comparison to non-radio-labelled standards. Confirmation was by TLC. The nature of radioactivity in the sodium hydroxide traps was investigated by barium chloride precipitation. Radioactivity in polyurethane bungs was quantified by soaking in acetonitrile followed by LSC.

## II. RESULTS AND DISCUSSION

Results for the full mass balance and characterisation of radioactivity in the water are shown in Table B.8.2.1.2-3. Recoveries of individual samples were  $\geq 96.2\%$  AR in all cases. In all cases, degradation of daminozide was slow, with the majority of radioactivity being associated with parent daminozide in the aqueous phase. However, greater degradation was shown in irradiated samples in the sterilised natural water than in the sterilised pure water. In this test, the metabolite Unknown-1 reached a maximum concentration of 2.4% AR, and Unknown-2 a maximum of 5.2% AR. The amount of radioactivity associated with the 2 N NaOH traps was 18.5% AR, which was shown to be  $^{14}\text{CO}_2$  by barium chloride precipitation.

In the irradiated pure water samples, degradation of parent daminozide was much slower. The maximum concentration of Unknown-1 was 2.6% AR at study termination, and Unknown-2 was not detected throughout the study duration. Concentrations of  $^{14}\text{CO}_2$  never exceeded 0.2% AR.

For the sterilised dark control samples degradation was slow, and it can be concluded that daminozide is essentially stable under such conditions.

**Table B.8.2.1.2-3: Distribution of radioactivity in water following application of [ $^{14}\text{C}$ ]-daminozide**

Fraction	Sample Time (Days after treatment)						
	0	1	4	7	10	13	15
<b>Sterilised Natural Water - Irradiated</b>							
<b>Total</b>	<b>103.6</b>	<b>97.8</b>	<b>96.8</b>	<b>97.5</b>	<b>97.9</b>	<b>96.5</b>	<b>99.0</b>
Water	103.6	97.8	95.0	92.4	90.7	84.2	80.5
Daminozide	101.8	93.8	92.4	86.4	84.8	78.1	74.7
Unknown-1	1.6	1.9	0.5	2.0	2.4	0.0	0.1
Unknown-2	ND	1.5	ND	3.6	3.5	4.3	5.2
Other degradates*	0.1	0.7	2.1	0.4	0.0	1.9	0.5
2 M NaOH traps <sup>†</sup>	NA	0.0	1.8	5.1	7.2	12.3	18.5
<b>Sterilised Natural Water – Dark Control</b>							
<b>Total</b>	<b>-</b>	<b>-</b>	<b>-</b>	<b>-</b>	<b>-</b>	<b>-</b>	<b>98.2</b>
Water	-	-	-	-	-	-	98.2
Daminozide	-	-	-	-	-	-	95.8
Unknown-1	-	-	-	-	-	-	0.8
Unknown-2	-	-	-	-	-	-	1.3
Other degradates*	-	-	-	-	-	-	0.3
<b>Sterilised Pure water - Irradiated</b>							
<b>Total</b>	<b>98.4</b>	<b>97.9</b>	<b>96.2</b>	<b>98.6</b>	<b>97.2</b>	<b>98.4</b>	<b>97.5</b>
Water	98.4	97.9	96.2	98.5	97.1	98.4	97.3
Daminozide	96.7	96.3	93.7	96.2	94.7	96.6	94.4
Unknown-1	0.8	0.6	2.4	1.9	2.3	1.8	2.6
Unknown-2	ND	ND	ND	ND	ND	ND	ND



Fraction	Sample Time (Days after treatment)						
	0	1	4	7	10	13	15
Other degradates*	0.8	0.9	0.1	0.4	0.1	<0.1	0.2
Foam Bung†	NA	0.0	0.0	0.1	0.0	0.0	0.0
2 M NaOH traps‡	NA	0.0	0.0	0.0	0.1	0.0	0.2
<b>Sterilised Pure water – Dark Control</b>							
<b>Total</b>	-	-	-	-	-	-	<b>98.5</b>
Water	-	-	-	-	-	-	98.5
Daminozide	-	-	-	-	-	-	96.8
Unknown-1	-	-	-	-	-	-	1.6
Unknown-2	-	-	-	-	-	-	ND
Other degradates*	-	-	-	-	-	-	0.1

NA = Not Analysed

\* Other degradates includes unresolved background radioactivity

† No radioactivity was detected in foam bungs, ethanediol or xylene traps

‡ No radioactivity was detected in ethanediol or xylene traps

### III. CONCLUSION

Degradation of daminozide in an irradiated sterilised natural water was slow, and in sterilised and pure water was very slow. In the sterilised natural water, 75% AR was unchanged daminozide at study termination, with 5.2% AR as an unknown metabolite (Unknown-2), and 18.5% AR as CO<sub>2</sub>. In the irradiated pure water, Unknown-2 was not detected throughout the study duration, and concentrations of <sup>14</sup>CO<sub>2</sub> never exceeded 0.2% AR. Daminozide was essentially stable in the sterilised dark control samples.

#### RMS comments and conclusion

Study is considered acceptable. The kinetic evaluation performed for the aqueous photolysis study of Brice and Scholey (2006) is presented in the section B.8.2.2.3 (study of Hilton and Callow, 2016).

#### B.8.2.1.3 Indirect photochemical degradation

In accordance with Comm. Reg. (EU) No. 283/2013 which sets out the active substance data requirements in accordance with Comm. Reg. (EC) No. 1107/2009, indirect photochemical degradation studies may be submitted where there are indications from other available data that route and rate of degradation in water can be significantly influenced by indirect photodegradation. This does not apply for daminozide, and therefore further data are not required. However, the study of Brice and Scholey (2006), summarised at B.8.2.1.2 above, was performed with sterilised natural water. The study showed that daminozide is degraded slowly under such conditions and that aqueous photolysis is unlikely to play a significant role in natural systems.

**B.8.2.2 Route and rate of biological degradation in aquatic systems****B.8.2.2.1 "Ready biodegradability"**

<b>Reference:</b>	<b>Ritter, A. (1989a)</b> Ready Biodegradability, Modified OECD Screening Test for Alar Technical (RCC 227384)
Report No.:	A.8.1.19
Document No:	-
Guideline:	OECD 301E
GLP:	Yes
<b>Previous evaluation:</b>	In DAR (1999)

Test compound: ALAR technical, i. e. N-dimethylaminosuccinamic acid

Batch: SI 1952

Purity: 99%

**Summary:**

The ready biodegradability of daminozide was determined in accordance with the OECD 301E guidelines. An inoculum was derived from the activated sludge from a domestic waste-water sewage plant and was added to the incubation vessels at nominal concentrations of 41.2 and 54.6 mg/L. After 28 days incubation in the dark at 21 – 22.5°C, 82% degradation was observed. As the degradation is shown to be > 70%, daminozide is considered as readily biodegradable.

Test organism: Microorganisms from a domestic waste water sewage plant (ARA Sissach/Switzerland)

Apparatus: 50 ml Erlenmeyer flasks loosely covered with an aluminium foil

Test medium: The test medium (30 ml per flask) was prepared according to the OECD Guideline 301 E.

Test concentration: The test article was dissolved in the test medium at a concentration of 41.2 and 54.6 mg/l corresponding to 18.4 and 25 mg DOC/l. The concentration of the standard (ANILINE) corresponded to theoretical amounts of 19.4 and 20.1 mg DOC/l. Except for one additional untreated inoculated control, all samples were run in duplicate.

### Test conditions

The study was run at 21 - 22.5 °C protected from light. The inoculated flasks were incubated in a shaking water bath.

### Sampling

Per sampling interval, two flasks were taken and analysed for DOC in duplicate. Samples were taken at day 0 (treatment day), 7, 14, 21, 27 and 28 of the incubation period. Water evaporation losses were compensated by adding bidistilled water.

### Analyses

Sample Preparation:

Samples were centrifuged or filtered (pore size: 0.2 micrometres) and thereafter directly analysed.

If analyses were performed later, 0.05 ml of a 1 % HgCl<sub>2</sub> solution per 10 ml filtrate were added.

Thereafter, the samples were stored for 24 hours at 2-4 °C or for a longer period of time at -18 to -20 °C (Directive 84/449 EEC).

DOC-Analyses:

DOC-analyses were performed with the various filtrates using a Technicon Carbon Analyser equipped with an Automatic Data Processing System.

**Table B.8.2.2.1-1: % DOC removal (test article)**

Test Set No.	7 d	14 d	21 d	27 d	28 d
1	68.3	74.2	74.8	83.3	80.5
2	10.1	75.3	72.8	80.9	83.5
mean	39.2	74.8	73.8	82.1	82.0

**Table B.8.2.2.1-2: % DOC removal (standard compound ANILINE)**

Test Set No.	7 d	14 d	21 d	27 d	28 d
3	98.1	99.1	95.9	97.5	98.4
4	98.7	95.7	93.8	94.6	98.7
mean	98.4	97.4	94.9	96.1	98.6

### Conclusion:

The test article, ALAR TECHNICAL, was degraded under the test conditions within 28 days to 82 %.

The standard compound, ANILINE, was degraded within 14 days to 97.4 %.

### RMS comments and conclusion

Study is considered acceptable. Daminozide is readily biodegradable.

**B.8.2.2.2 Aerobic mineralisation in surface water**

<b>Reference:</b>	<b>Button, S. (2015)</b> Daminozide: Aerobic Mineralisation in Surface Water.
Report No.:	FDD0108
Document No:	-
Guideline:	OECD 309: Aerobic Mineralisation in Surface Water – Simulation Biodegradation Test (April 2004)
GLP:	Yes
<b>Previous evaluation:</b>	Submitted for the purpose of renewal

Test compound: Daminozide, labelled in the [<sup>14</sup>C-dimethylamino]- position

Lot number: 13BLY027

Radiochemical purity: 93.8%

Specific radioactivity: 25 mCi/mmol

**Executive Summary:**

The aerobic metabolism of [<sup>14</sup>C-dimethylamino]-daminozide was studied in a single sieved and filtered surface water system under aerobic conditions in the laboratory. Surface water samples, with a measured pH of 8.14, were freshly collected from the River Ouse, UK, and treated with [<sup>14</sup>C]-daminozide at nominal application rates of 2 µg/L and 10 µg/L. Test systems were maintained with constant agitation, at 20 ± 2°C and in darkness, for up to 30 days. For the HPLC characterisation of radioactivity, duplicate treated samples for each sample time were sealed in individual vessels and incubated until sample sacrifice. Additional single treated samples for each sample time were used for the measurement of volatile degradation products. Air was drawn through the volatile test vessels and outgoing air was passed through a series of traps for the trapping of CO<sub>2</sub> and other volatiles.

Radioactivity in surface water samples was measured by liquid scintillation counting (LSC), and characterisation was performed by HPLC with radio- and UV detection. LC-MS was conducted on selected surface water samples for the identification and confirmation of degradates and daminozide. An LOD of 2.5% AR in the 2 µg/L samples and 0.7% AR in the 10 µg/L samples was calculated for the analysis of total radioactivity. An LOQ of 10% AR was set for both LSC and HPLC characterisation.

Total mean recoveries of radioactivity (aqueous solution and volatiles) for samples treated at a concentration of 2 µg/L were between 77.7 – 100.4% AR. Recoveries for samples treated at 10 µg/L were between 83.0 – 99.8% AR. A minority of samples displayed mass balances < 90% AR (the final sample for the 10 µg/L study and the final two samples for the 2 µg/L tests displaying mean recoveries of < 90% AR). This is likely to be due to losses of <sup>14</sup>CO<sub>2</sub> due to its very high formation. The slightly low recovery of these few samples does not affect the reliability of the study.

Daminozide degraded via an unknown polar component (mean maximum of 75.7% AR at 2 µg/L and 35.4% AR at 10 µg/L, 2 and 3 days after treatment) to the terminal degradation product, CO<sub>2</sub> (mean maximum concentrations of 57.3% AR at 2 µg/L and 55.9% AR at 10 µg/L in individual samples 30 days after treatment). In order to attempt to identify the polar component, exaggerated rate (*ca* 200µg/L) samples were incubated under study conditions and monitored periodically to follow the formation of the degradate of interest. Following incubation

for 15 days the samples were analysed by LC/MS. However, no conclusive results were obtained. ~~Further work is ongoing to identify this metabolite.~~

Parent daminozide was degraded to concentrations <LOQ in both replicates by 1 day in the 2 µg/L test and 2 days in the 10 µg/L test. Degradation rates were calculated in accordance with FOCUS Kinetics guidance. FOMC kinetics returned the most appropriate fits for daminozide, and returned DT<sub>50</sub> values of 0.13 - 0.15 days with corresponding DT<sub>90</sub> values of 0.42 - 1.7 days. SFO kinetics provided the best fits for the polar metabolite; DT<sub>50</sub> values of 1.6 – 4.5 days were calculated.

## I. MATERIAL AND METHODS

Daminozide, labelled in the [<sup>14</sup>C-dimethylamino]- position, was applied to freshly sampled water from the River Ouse, UK. The river water was filtered through a 0.2 mm sieve and a coarse filter paper (GF/A), characterized, and used within 1 day of collection. Characteristics of the water are presented in Table B.8.2.2.2-1. River water (100 mL) was dispensed into individual 500 mL test flasks, and samples incubated in the dark at 20 ± 2°C for up to 30 days. For the HPLC characterisation of radioactivity, duplicate treated samples for each individual sample time were sealed in vessels and incubated until sample sacrifice. Additional single treated samples for each sample time were used for the measurement of volatile degradation products. Air was drawn through the volatile test vessels and outgoing air was passed through a series of traps (containing ethyl digol and 1 M potassium hydroxide) for the trapping of organic volatiles and CO<sub>2</sub>. An additional two samples were incubated in the same manner as the volatile samples to determine mass balance at study termination.

Samples were continuously and gently stirred to maintain particulate and micro-organisms in suspension and to facilitate oxygen transfer from the headspace to the aqueous phase, and maintain aerobic conditions. For sterile samples, test water and vessels were additionally sterilized with an autoclave.

**Table B.8.2.2.2-1: Water parameters**

Origin/Source		River Ouse, UK
Temperature <sup>1</sup>	[°C]	5.7
pH <sup>1</sup>	-	8.14
Oxygen saturation <sup>1</sup>	[%]	86
Total organic carbon (TOC) <sup>2</sup>	[mg/L]	5.3
Dissolved organic carbon (DOC) <sup>2</sup>	[mg/L]	5.2

<sup>1</sup> measured at field sampling.

Individual test flasks for two test systems were set-up to investigate the aerobic mineralization of daminozide at two concentrations; 2 µg/L and 10 µg/L. Additional vessels were set up for the determination of the microbiological activity at the start and end of the incubation period and the measurement of the pH, oxygen content and radioactivity background levels in the surface water.

Samples of surface water were treated with reference substance  $^{14}\text{C}$  sodium benzoate and were arranged in flow-through systems designed to trap volatile radiolabelled  $^{14}\text{CO}_2$  in order to determine the microbial viability of the test system. In addition, two sterilized samples for each application rate were incubated. Reference substance samples and sterilized samples were incubated and maintained in the same manner as the non-sterilized samples, with the exception that samples were only analysed at a single timepoint (day 14 for reference substance samples and day 30 for sterilized samples). Untreated control samples were also incubated under the same conditions. Applications of the test substance were made to samples at a verified concentration of 2.0  $\mu\text{g/L}$  and 10.0  $\mu\text{g/L}$ . Duplicate samples treated with the test item were taken immediately after application, and triplicate samples (duplicate sealed vessels and a single vessel with volatile traps) at study termination after 30 days, and at 7 intermediate time-points. Duplicate sodium benzoate treated test systems were removed and analyzed after 14 days. At each sampling interval the pH and oxygen content of blank samples was determined. Radioactivity in surface water samples was measured by liquid scintillation counting (LSC), and characterisation was performed by HPLC with radio- and UV detection. LC-MS was conducted on selected surface water samples for the identification and confirmation of degradates and daminozide. Aliquots of volatile trap solutions were analysed by LSC at sample sacrifice. The surface water was then acidified with concentrated HCl to pH 2-3, and the test vessel re-connected to the trapping system. After at least two hours aliquots from the trapping solution were re-analysed by LSC. The presence of  $^{14}\text{CO}_2$  was confirmed by barium chloride precipitation. An LOD of 2.5% AR in the 2  $\mu\text{g/L}$  samples and 0.7% AR in the 10  $\mu\text{g/L}$  was calculated for the analysis of radioactivity by LSC. The LOQ of the LSC and HPLC analysis was set at 10% AR.

The decline of daminozide and its unidentified polar metabolite was kinetically evaluated in accordance with FOCUS Kinetics guidance (FOCUS, 2006), considering SFO and FOMC kinetics. Residues between LOQ and LOD were considered as  $0.5 \times (\text{LOQ} + \text{LOD})$ , while the first residue  $<\text{LOD}$  was considered as  $0.5 \times \text{LOD}$  and subsequent values were omitted from the kinetic evaluation. For daminozide at both concentrations, the radiochemical purity value was used in lieu of the measured zero-time values. Degradation of the polar metabolite was considered from the peak down rather than as part of a degradation scheme. Considering the very rapid degradation of parent daminozide, and the fact that peak concentrations of the metabolite occur after daminozide concentrations are  $<\text{LOQ}$ , this is appropriate to calculate reliable  $\text{DT}_{50}$  values.

## II. RESULTS AND DISCUSSION

The oxygen content varied between 83 and 96% oxygen saturation, and was indicative of an aerobic system for the full study duration. The pH of the test system showed small variations of 8.09 to 8.73 over the full study duration. After 14 days incubation, the reference substance [ $^{14}\text{C}$ ]-sodium benzoate was present at concentrations of 10% AR and 12.5% AR in the aqueous phase, with total  $^{14}\text{CO}_2$  comprising 86.6 – 93.4% AR. The reference study results confirmed the microbially active nature of the test systems. The recovery and distribution of the radioactivity at the different sampling times is shown in Table B.8.2.2.2-2 to Table B.8.2.2.2-5.

**Table B.8.2.2.2-2: Balance of radioactivity (% AR) after the application of [<sup>14</sup>C]-daminozide at a rate of 2 µg/L in the single ‘volatile samples’**

	Incubation Time (days)								
	0	0.08	0.25	1	2	3	7	14	30*
<b>Aqueous Phase</b>	-	<b>98.5</b>	<b>98.0</b>	<b>71.0</b>	<b>43.7</b>	<b>56.1</b>	<b>42.3</b>	<b>36.3</b>	<b>28.4</b>
<b>Total <sup>14</sup>CO<sub>2</sub></b>	-	<b>nd</b>	<b>2.4</b>	<b>25.8</b>	<b>54.9</b>	<b>43.0</b>	<b>56.0</b>	<b>41.4</b>	<b>57.3</b>
Pre-acidification	-	nd	nd	3.5	32.9	33.9	45.5	31.8	47.3
Post acidification	-	nd	2.4	22.3	22.0	9.1	10.5	9.6	10.0
<b>Total</b>		<b>98.5</b>	<b>100.4</b>	<b>96.8</b>	<b>98.6</b>	<b>99.1</b>	<b>98.3</b>	<b>77.7</b>	<b>85.7</b>

\* Day 30 values are mean values from 3 replicates – single volatile sample and 2 additional mass balance samples  
No other volatile components were observed.

**Table B.8.2.2.2-3: Characterisation of radioactivity (% AR) in surface water after the application of [<sup>14</sup>C]-daminozide at a rate of 2 µg/L in the duplicate ‘sealed samples’**

		Incubation Time (days)								
		0	0.08	0.25	1	2	3	7	14	30
<b>Aqueous Phase</b>	Rep 1	102.9	100.2	100.5	86.7	100.4	90.3	73.9	48.4	37.5
	Rep 2	100.9	97.8	97.8	86.8	87.1	82.9	67.6	43.7	48.7
	<b>Mean</b>	<b>101.9</b>	<b>99.0</b>	<b>99.2</b>	<b>86.8</b>	<b>93.8</b>	<b>86.6</b>	<b>70.8</b>	<b>46.1</b>	<b>43.1</b>
<b>Daminozide</b>	Rep 1	81.4	59.7	29.5	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	Rep 2	89.9	60.5	17.7	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
	<b>Mean</b>	<b>85.7</b>	<b>60.1</b>	<b>23.6</b>	<b>&lt;LOD</b>	<b>&lt;LOD</b>	<b>&lt;LOD</b>	<b>&lt;LOD</b>	<b>&lt;LOD</b>	<b>&lt;LOD</b>
<b>Polar Component</b>	Rep 1	<LOD	<LOD	23.0	68.9	88.2	54.0	50.6	<LOQ	<LOQ
	Rep 2	<LOD	<LOD	44.8	30.6	63.1	47.3	20.8	13.4	<LOQ
	<b>Mean</b>	<b>&lt;LOD</b>	<b>&lt;LOD</b>	<b>33.9</b>	<b>49.8</b>	<b>75.7</b>	<b>50.7</b>	<b>35.7</b>	<b>&lt;LOQ*</b>	<b>&lt;LOQ</b>

LOQ = 10% AR

LOD = 2.5% AR

\* Mean concentration calculated assuming replicates <LOQ = ½ x LOQ, or 5% AR.

**Table B.8.2.2.2-4: Balance of radioactivity (% AR) after the application of [<sup>14</sup>C]-daminozide at a rate of 10 µg/L in the single ‘volatile samples’**

	Incubation Time (days)								
	0	0.08	0.25	1	2	3	7	14	30*
<b>Aqueous Phase</b>	-	<b>98.8</b>	<b>98.3</b>	<b>92.9</b>	<b>53.2</b>	<b>48.0</b>	<b>57.7</b>	<b>51.2</b>	<b>27.1</b>
<b>Total <sup>14</sup>CO<sub>2</sub></b>	-	<b>nd</b>	<b>0.7</b>	<b>5.4</b>	<b>43.2</b>	<b>51.8</b>	<b>36.8**</b>	<b>41.2</b>	<b>55.5**</b>
Pre-acidification	-	nd	nd	1.6	22.9	36.8	31.2	35.4	49.4
Post acidification	-	nd	0.7	3.8	20.3	15.0	6.2	5.8	6.5
<b>Total</b>	-	<b>98.8</b>	<b>99.0</b>	<b>98.3</b>	<b>96.4</b>	<b>99.8</b>	<b>95.1</b>	<b>92.4</b>	<b>83.0</b>

\* Day 30 values are mean values from 3 replicates – single volatile sample and 2 additional mass balance samples

\*\* In samples taken at day 7 and day 30 0.6% AR and a mean of 0.4% AR was additionally observed in the ethyl digol volatile trap.

**Table B.8.2.2.2-5: Characterisation of radioactivity (% AR) in surface water after the application of [<sup>14</sup>C]-daminozide at a rate of 10 µg/L in the duplicate ‘sealed samples’**

		Incubation Time (days)								
		0	0.08	0.25	1	2	3	7	14	30
<b>Aqueous Phase</b>	Rep 1	99.2	97.2	97.9	93.8	90.9	86.3	65.0	37.9	35.5
	Rep 2	100.8	97.8	98.4	95.5	87.8	85.3	72.0	52.0	45.6
	<b>Mean</b>	<b>100.0</b>	<b>97.5</b>	<b>98.2</b>	<b>94.7</b>	<b>89.4</b>	<b>85.8</b>	<b>68.5</b>	<b>45.0</b>	<b>40.6</b>
<b>Daminozide</b>	Rep 1	66.5	63.3	37.9	<LOQ	<LOD	<LOD	<LOD	<LOD	<LOD
	Rep 2	48.7	53.9	34.7	36.7	<LOD	<LOD	<LOD	<LOD	<LOD
	<b>Mean</b>	<b>57.6</b>	<b>58.6</b>	<b>36.3</b>	<b>20.9*</b>	<b>&lt;LOD</b>	<b>&lt;LOD</b>	<b>&lt;LOD</b>	<b>&lt;LOD</b>	<b>&lt;LOD</b>
<b>Polar Component</b>	Rep 1	<LOD	<LOD	<LOD	<LOD	28.8	44.4	<LOQ	<LOQ	<LOQ
	Rep 2	<LOD	<LOD	<LOD	<LOD	27.0	26.3	<LOQ	<LOQ	<LOQ
	<b>Mean</b>	<b>&lt;LOD</b>	<b>&lt;LOD</b>	<b>&lt;LOD</b>	<b>&lt;LOD</b>	<b>27.9</b>	<b>35.4</b>	<b>&lt;LOQ</b>	<b>&lt;LOQ</b>	<b>&lt;LOQ</b>

LOQ = 10% AR

\* Mean concentration calculated assuming replicates <LOQ = ½ x LOQ, or 5% AR

Total recoveries from volatile samples and mass balance samples declined from 98.5 – 98.8% AR after 2 hours, to mean values of 83.0 – 85.7% AR at study termination. The majority of the samples displayed recoveries of > 90% AR, with only the final sample for the 10 µg/L study and the final two samples for the 2 µg/L tests displaying mean recoveries of < 90% AR. This is likely to be due to losses of <sup>14</sup>CO<sub>2</sub> due to its very high formation. The slightly low recovery of these few samples does not affect the reliability of the study. Total <sup>14</sup>CO<sub>2</sub> concentrations increased throughout the study duration reaching mean maximum concentrations of 55.5 – 57.3% AR at study termination. No other volatile components were observed.

HPLC analysis of surface water resolved a major polar component which reached maximum mean concentrations of 75.7% AR and 35.4% AR in the 2 µg/L and 10 µg/L tests, respectively. In order to attempt to identify this component, exaggerated rate (*ca* 200µg/L) samples were incubated under study conditions and monitored periodically to follow the formation of the degradate of interest. Following incubation for 15 days the samples were analysed by LC/MS. However, no conclusive results were obtained. ~~Further work is ongoing to identify this metabolite.~~

Daminozide degraded rapidly at both application rates with all replicates displaying daminozide concentrations < LOQ 1-2 days after treatment. Degradation rates were calculated in accordance with FOCUS Kinetics guidance. The software ModelMaker (version 4.0) was used. FOMC kinetics returned the most appropriate fits for daminozide, and returned DT<sub>50</sub> values of 0.13 days and 0.15 days for the 2.0 µg/L and 10.0 µg/L application rates respectively. Corresponding DT<sub>90</sub> values were 0.42 days and 1.7 days. SFO kinetics provided the best fits for the polar metabolite; DT<sub>50</sub> values of 1.6 – 4.5 days were calculated. Full statistics for the kinetic evaluations of daminozide and its polar metabolite are presented in Table B.8.2.2.2-6.



Table B.8.2.2.2-6: Summary of the results of the kinetic determinations for daminozide

Model	Parameter	2 µg/L application rate		10 µg/L application rate	
		Daminozide	Polar Metabolite	Daminozide	Polar Metabolite
SFO	$\chi^2$ error (%)	1.0	<b>12.7</b>	17.5	<b>23.7</b>
	k	5.449 ± 0.363	<b>0.153 ± 0.044</b>	3.440 ± 1.001	<b>0.440 ± 0.201</b>
	DT <sub>50</sub>	0.13	<b>4.5</b>	0.20	<b>1.6</b>
	DT <sub>90</sub>	0.42	<b>15.1</b>	0.67	<b>5.2</b>
	t-test	Significant at 0.1%	Significant at 1%	Significant at 1%	Significant at 5%
FOMC	$\chi^2$ error (%)	<b>1.2</b>	12.3	<b>10.2</b>	29.6
	$\alpha$	<b>151.7 ± 1322.9</b>	1.59E12 ± 1.16E15	<b>8.347 ± 0.439</b>	1.090 ± 1.052
	$\beta$	<b>27.74 ± 240.2</b>	3.62E12 ± 2.61E15	<b>0.118 ± 0.111</b>	3.575 ± 5.823
	DT <sub>50</sub>	<b>0.13</b>	1.6	<b>0.15</b>	3.2
	DT <sub>90</sub>	<b>0.42</b>	5.2	<b>1.7</b>	26.0
	t-test	$\alpha$ , $\beta$ significant at >10%	$\alpha$ , $\beta$ significant at >10%	$\alpha$ significant at 0.1%, $\beta$ >10%	$\alpha$ significant at 0.1%, $\beta$ >10%

Fits presented in bold font are considered the best fits.

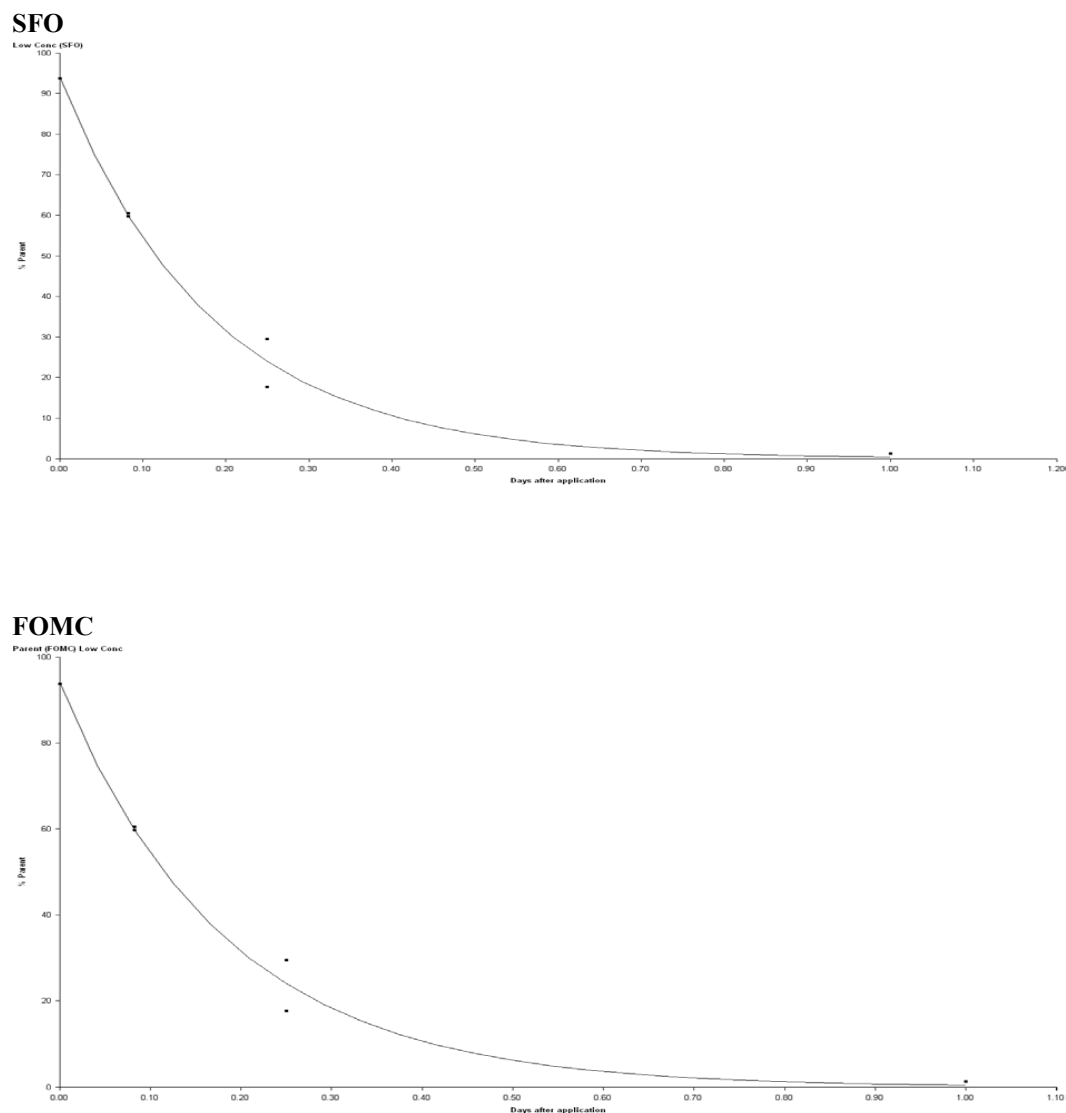
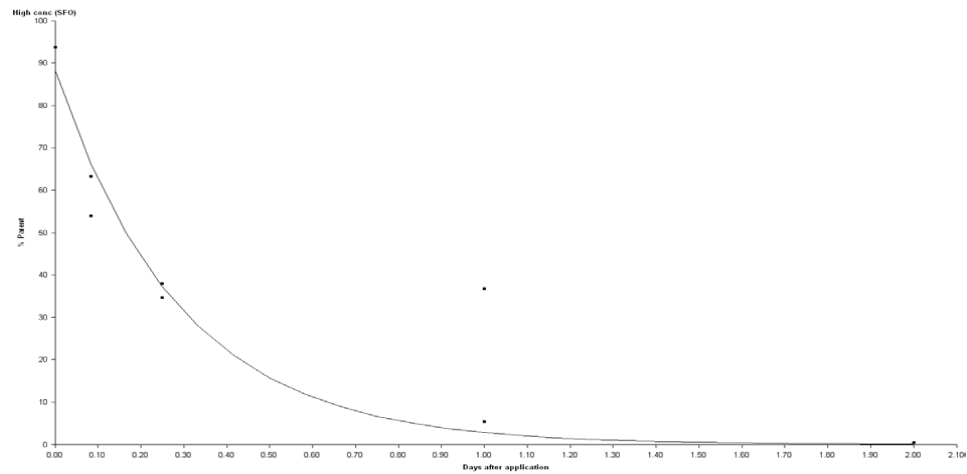
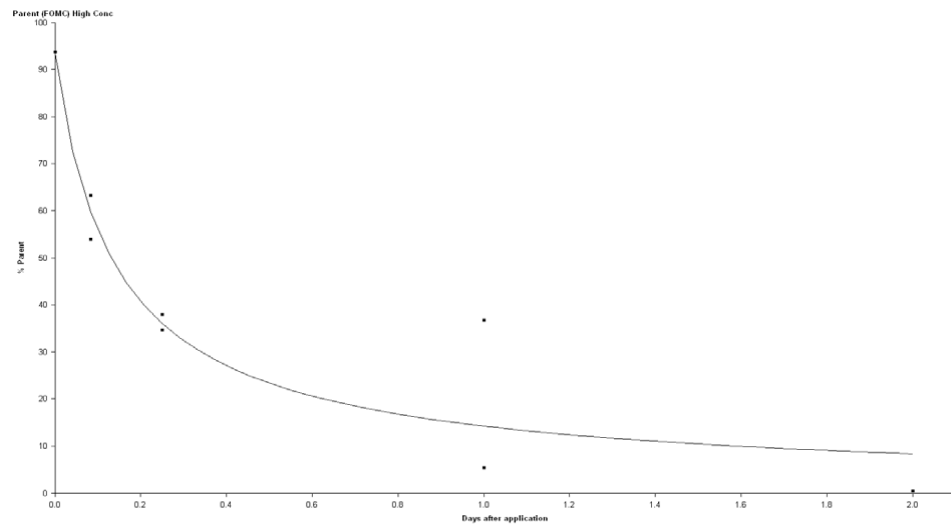
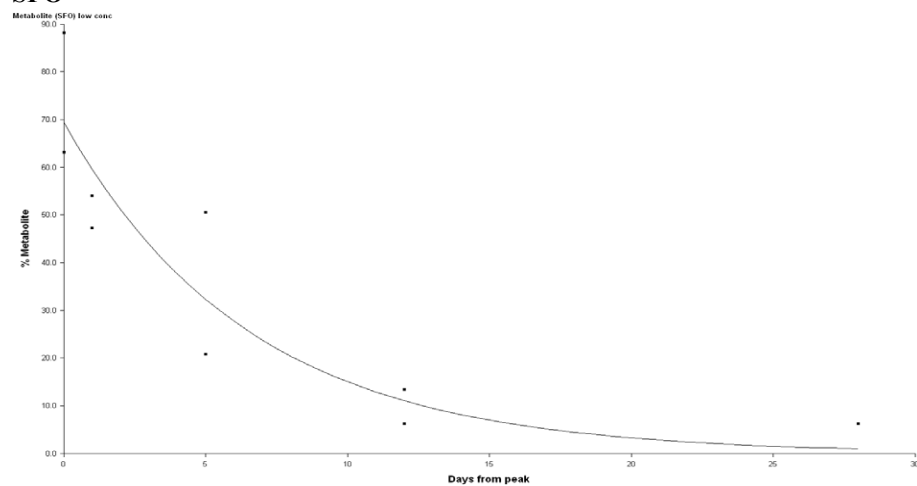
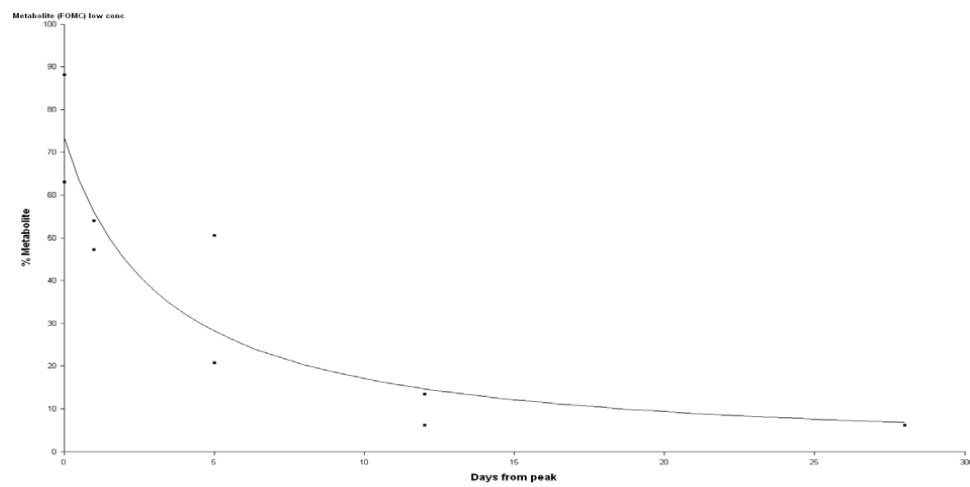
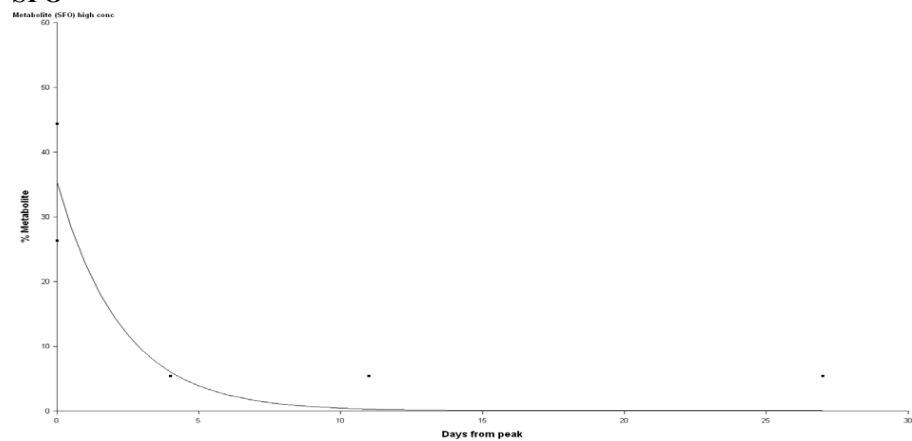
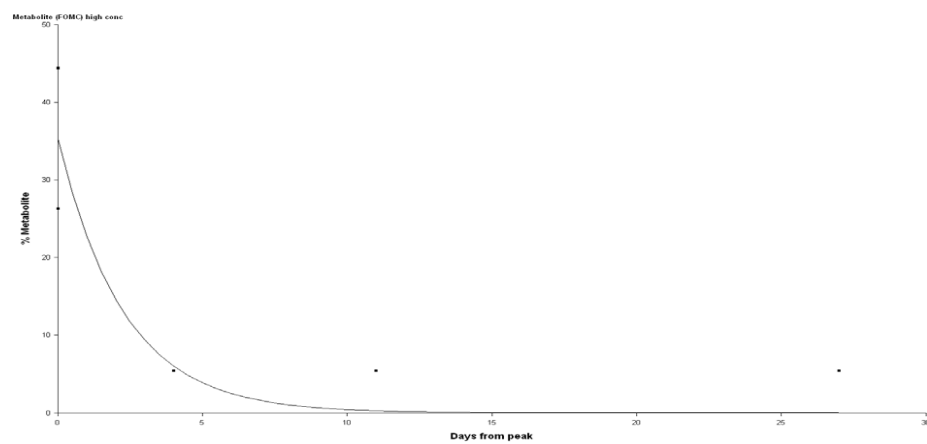


Figure B.8.2.2.2-1: Decline of daminozide in surface water treated at 2 µg/L

**SFO****FOMC**

**Figure B.8.2.2-2: Decline of daminozide in surface water treated at 10 µg/L**

**SFO**

**FOMC****Figure B.8.2.2.2-3: Decline of unknown metabolite in surface water treated at 2 µg/L****SFO****FOMC****Figure B.8.2.2.2-4: Decline of unknown metabolite in surface water treated at 10 µg/L**

### III. CONCLUSION

Daminozide degraded rapidly under the conditions of the test, with FOMC  $DT_{50}$  values of 0.13 – 0.15 days, and  $DT_{90}$  values of 0.42 – 1.7 days. The degradation of daminozide was via an unidentified polar metabolite (maximum mean formation 35.4 – 75.7% AR), which itself degraded rapidly with SFO  $DT_{50}$  values of 1.6 – 4.5 days, to the terminal degradation product  $CO_2$  (max formation 55.9 – 57.3% AR at study termination). No other metabolites were observed.

#### RMS comments and conclusion:

Limit of detection was 2.5% AR at low dose, it should be at least 1% according to OECD test guideline.

Total mass balance at two last sampling points for low dose samples were below 90% (77.7% after 14 days only). It can be caused by losses of  $CO_2$  but also by the methanol. Significant amount of applied radioactivity in aqueous phase was not identified. For example at day 0 total recovery in aqueous phase (low dose) was 101.9% AR and only 85.7% AR was identified as daminozide, it is not clear what was the remaining radioactivity. The same problem was with high dose study where half of radioactivity was identified or even less, e.g. at day 0.25 only 36.3% AR was daminozide and no other metabolite was identified.

FOMC for parent compound at low dose did not improve the fit, alpha and beta contain zero, SFO would be more appropriate. FOMC for high dose samples gave better visual fit and  $\chi^2$  is below 15%.

Kinetic degradation of unknown metabolite - last two points out of five had low recoveries at low dose samples. SFO kinetics fit for unknown metabolite at high dose samples is visually pure,  $\chi^2$  is >15%. FOMC kinetics did not improve the fit,  $\chi^2$  is very high and beta parameter contains zero.

Due to several deviations mentioned above, results from this study are not considered reliable.

#### Statement submitted by the notifier to unknown metabolite:

*The mineralisation of daminozide in surface water under aerobic conditions was investigated in the study of Button, 2015. Daminozide degraded rapidly under the conditions of the test, with FOMC  $DT_{50}$  values of 0.13 – 0.15 days and corresponding  $DT_{90}$  values of 0.42 – 1.7 days. The degradation of daminozide was via an unidentified polar metabolite (maximum 75.7% AR) which itself degraded rapidly ( $DT_{50}$  1.6 – 4.5 days) to the terminal degradation product  $CO_2$  (max formation 55.9 – 57.3% AR at study termination). Attempts to identify this polar metabolite were not successful due to the low concentration and the high polarity of the metabolite. However, similar to the water/sediment study, the polar metabolite was also rapidly and instantaneously formed in the water phase. The HPLC analyses show that the same metabolic pattern was observed in all three studies. Therefore, it can be concluded that the same metabolite is to be expected in the aerobic mineralisation in surface water study as in the water phase from the water sediment study.*

*Within Button (2015) study, attempts were made to generate the unknown metabolite for further identification work using a high dose. The degradation was slower due to the limited microbial activity of the test system used. However, the same metabolic pattern was observed.*

*The high dose generated water sample in the same system was sent to Agrosience for further identification and comparison with the soil metabolite. The sample was analysed by two highly different HPLC methods using the methods used in the Möndel, 2015, study and also with the soil extract polar metabolite identified as methanol. Results show that it is the same metabolite showing the same behaviour and the same retention time as the soil metabolite and therefore it corresponds to methanol. The same metabolic pattern was confirmed from the water sediment study based on similarity of the HPLC chromatograms.*

#### **RMS comments:**

The identification of unknown metabolite M1 is very briefly reported and is considered not sufficient. The method should be described in details and chromatograms should be provided.

The method used in the Möndel, 2015, study for identification of unknown metabolite was not successful. The unknown metabolite was finally identified in the study by DeMaio (2015) comparing the unknown peak with the reference standard. This should be also confirmed by other specific method.

In conclusion, the RMS is of opinion that it was not clearly demonstrated that unknown metabolite is methanol. It should be clearly demonstrated that unknown peak is methanol and the remaining radioactivity should be identified.

#### **B.8.2.2.3 Water/sediment study**

<b>Reference:</b>	<b>De Vette, H.Q.M., van Es, C. (2002)</b> A study on the degradation of [ <sup>14</sup> C] daminozide in two water/sediment systems.
Report No.:	TNO study number 01-2482/01, 2002
Document No:	-
Guideline:	OECD 308 (draft)
GLP:	Yes
<b>Previous evaluation:</b>	In Addendum II (2002)

Test compound: [<sup>14</sup>C]-daminozide

Lot number: CSL-99-867-38-23

Radiochemical purity: 93.8%

Specific radioactivity: 22.4 mCi/mmol

#### **Executive Summary:**

The study was performed with two water/sediment systems; collected from locations in the Netherlands. System 1 was collected from the TNO ditch (sediment pH 7.5, OC 14.9%; and water pH 7.1) and System 2 (sediment pH 7.6, OC 4.9%; and water pH 6.4) was sampled from the Kromme Rijn river. Following application of 1 mg/L of [<sup>14</sup>C]-daminozide, individual test systems were connected to a series of traps and incubated at 20°C in the dark for

a maximum of 21 days. Moist air was drawn through the test vessel and outgoing air was passed through a series of traps for the trapping of CO<sub>2</sub> and other volatiles.

Several samples displayed mass balances <90% AR, however this is considered to be due to losses of <sup>14</sup>CO<sub>2</sub>, which is observed at concentrations of up to 38.9% AR, and therefore does not affect the validity of the study with respect to either the route or rate of degradation of daminozide.

Parent daminozide was completely degraded in both systems by 7 days after treatment. Formaldehyde, characterised by HPLC and comparison of retention times to those of a reference standard, was the only metabolite reported to be observed at concentrations > 5% AR, in either the water or sediment phases. Maximum concentrations of 17.0% AR, 9.5% AR and 24.1% AR in water, sediment and total system respectively were reported.

The study demonstrates that in natural water/sediment systems daminozide is likely to rapidly degrade to formaldehyde which in turn is degraded to CO<sub>2</sub>.

## I. MATERIAL AND METHODS

The study was performed with two water/sediment systems. System 1 was collected from the TNO ditch and System 2 was sampled from the Kromme Rijn river; both water/sediment systems were located in the Netherlands. Characteristics of the water/sediment systems are presented in Table below.

**Table B.8.2.2.3-1: Composition of the two water sediment systems**

	<b>System 1</b>	<b>System 2</b>
	<b>TNO Ditch</b>	<b>Kromme Rijn River</b>
<b>Sediment:</b>		
63 µm – 2 mm	60.1%	27.3%
2 µm - 63 µm	18.4%	41.6%
< 2 µm	21.5%	31.2%
pH (H <sub>2</sub> O)	7.9	8.0
pH (0.01 M CaCl <sub>2</sub> )	7.5	7.6
CEC (meq/ 100 g)	51.8	28.9
Organic Carbon (%)	14.9	4.9
<b>Water:</b>		
pH	7.1	6.4

System 1 was incubated as 100 g – 200 g dry weight sediment, and System 2 as 170 g - 230 g dry weight sediment in a total volume of 600 mL. Flasks were pre-incubated at 20°C in the dark for 10 days. Following application of 1 mg/L of [<sup>14</sup>C]-daminozide, applied to the water phase of the system, individual test systems were connected to a series of traps and incubated at 20°C in the dark for a maximum of 21 days. Moist air was drawn through the test vessel and outgoing air was passed through two potassium hydroxide traps for CO<sub>2</sub> trapping, a single sulphuric acid trap for other volatiles, and two paraffin traps for the trapping of methane. Carbon dioxide remaining in the aqueous phase was determined by acidification with hydrochloric acid, and collection in a 1.5 M KOH solution. The amount of radioactivity present in the trapping solutions was determined by LSC. Duplicate samples were collected immediately after application, at study termination, and at 6 intermediate time-points. Water and sediment was separated, and the sediment extracted by shaking in acetonitrile at room temperature. Subsequent extractions were performed by shaking in methanol and ultra-pure water until < 3% AR was observed in extracts. Radioactivity in both the aqueous and sediment phases was determined by LSC, and characterisation and

quantification was performed by HPLC equipped with radioactivity and UV detectors. Unextracted sediment residues were determined by combustion and LSC.

## II. RESULTS AND DISCUSSION

The recoveries of radioactivity in the TNO ditch and Kromme Rijn river systems are presented in Tables B.8.2.2.3-2 – B.8.2.2.3-3. Some samples display recoveries <90% AR, however this is considered to be due to losses of  $^{14}\text{CO}_2$ , which is observed at concentrations of up to 38.9% AR, and therefore does not affect the validity of the study with respect to either the route or rate of degradation of daminozide.

The characterisation of radioactivity in the aqueous phase and extracted sediment from the TNO ditch and Kromme Rijn river systems is presented in Tables B.8.2.2.3-4 and B.8.2.2.3-5. A full characterisation of extracted radioactivity was not performed for the final two sample times taken after 11 and 21 days. However, parent daminozide had completely degraded in both systems by 7 days after treatment.

Concentrations of the major metabolite formaldehyde were increasing at 7 days after treatment. However, extractable radioactivity concentrations determined at later time-points demonstrate that only small increases of formaldehyde concentrations would be possible in the sediment phase and whole system, while aqueous phase concentrations of formaldehyde must be lower than observed at 7 days. Based on the total extracted radioactivity, the maximum theoretical concentration of formaldehyde (assuming all extracted radioactivity in aqueous and sediment phase is formaldehyde only) would be observed in the day 11 sample in the Kromme Rijn system, and would be 12.4% AR in sediment and 27.6% AR in the whole system. Therefore, the maximum formation of formaldehyde is not significantly affected by the lack of characterisation of the 11 day and 21 day samples.

All other metabolites were observed at concentrations  $\leq 3.4\%$  AR, and because of the pattern of observed concentrations of the metabolites, and the concentrations of formaldehyde at 7 days (which comprised the vast majority of extracted radioactivity), they would not be anticipated to be formed at higher concentrations in subsequent samples.

**Table B.8.2.2.3-2: Distribution of radioactivity in the TNO water/sediment system (as mean % AR)**

Sample (days after treatment)	$^{14}\text{CO}_2$ traps	$^{14}\text{CO}_2$ dissolved	$^{14}\text{CO}_2$ total	$\text{H}_2\text{SO}_4$	paraffin	Aqueous phase	Sediment phase	Bound residue	Total Radioactivity
0	nd	nd	nd	nd	nd	86.9	2.9	3.8	93.5
7 hrs	0.1	7.2	7.3	0.0	0.0	75.6	3.5	7.3	93.7
21 hrs	1.7	16.6	18.3	0.0	0.0	57.3	4.5	13.8	93.9
2	3.4	20.5	23.9	0.0	0.0	31.2	6.9	15.4	77.4
4	4.9	22.3	27.3	0.0	0.0	30.2	6.5	17.6	81.5
7	13.0	25.4	38.4	0.0	0.0	17.0	7.1	18.3	80.8
11	13.2	24.1	37.3	0.0	0.0	15.0	8.6	18.5	79.4
21	17.5	19.5	37.0	0.0	0.0	11.5	6.1	21.5	76.1

*nd – not determined*

**Table B.8.2.2.3-3: Distribution of radioactivity in the Kromme Rijn water/sediment system (as mean % AR)**

Sample (days after treatment)	<sup>14</sup> CO <sub>2</sub> traps	<sup>14</sup> CO <sub>2</sub> dissolved	<sup>14</sup> CO <sub>2</sub> total	H <sub>2</sub> SO <sub>4</sub>	paraffin	Aqueous phase	Sediment phase	Bound residue	Total Radioactivity
0	nd	nd	nd	nd	nd	85.7	3.0	2.7	91.4
7 hrs	0.3	5.8	6.0	0.0	0.0	77.2	7.0	10.3	100.5
21 hrs	1.3	18.1	19.4	0.0	0.0	56.3	5.0	12.4	93.2
2	5.1	23.4	28.4	0.0	0.0	28.1	7.6	19.8	84.0
4	8.6	26.0	34.5	0.0	0.0	18.7	8.0	20.3	81.5
7	12.0	26.9	38.9	0.0	0.0	13.8	9.5	19.4	81.6
11	14.7	21.7	36.4	0.0	0.0	15.2	12.4	14.9	78.8
21	17.4	9.9	27.2	0.0	0.0	6.8	5.0	34.9	73.9

nd – not determined

**Table B.8.2.2.3-4: Characterisation of radioactivity in the aqueous phase and extracted from sediment of the TNO water/sediment system (as mean % AR)**

Sample (days after treatment)	Daminozide			Formaldehyde			Unidentified Metabolites*			Sum
	Water	Sediment	Total System	Water	Sediment	Total System	Water	Sediment	Total System	
0	85.5	2.7	88.2	3.2	0.2	3.3	0.0	0.0	0.0	91.6
7 hrs	71.0	0.0	71.0	2.5	3.5	6.0	2.1	0.0	2.1	79.1
21 hrs	48.3	2.1	50.4	9.0	2.3	11.3	0.0	0.0	0.0	61.7
2	15.7	0.5	16.3	15.4	6.4	21.8	0.0	0.0	0.0	38.1
4	15.8	0.0	15.8	14.4	6.5	20.9	0.0	0.0	0.0	36.7
7	0.0	0.0	0.0	17.0	7.1	24.1	0.0	1.1	1.1	25.2

nd – not detected

\* Unidentified Metabolites comprises two metabolites: Unidentified I was solely observed in the water phase, and Unidentified II was solely observed in the sediment phase.

**Table B.8.2.2.3-5: Characterisation of radioactivity in the aqueous phase and extracted from sediment of the Kromme Rijn water/sediment system (as mean % AR)**

Sample (days after treatment)	Daminozide			Formaldehyde			Unidentified I			Sum
	Water	Sediment	Total System	Water	Sediment	Total System	Water	Sediment	Total System	
0	77.6	3.0	80.6	6.4	0.0	6.4	3.4	0.0	3.4	90.4
7 hrs	72.9	6.7	79.7	4.3	0.3	4.6	0.0	0.0	0.0	84.2
21 hrs	45.2	3.4	48.6	11.2	1.6	12.7	0.0	0.0	0.0	61.3
2	15.5	0.5	16.0	12.6	6.3	18.9	0.0	0.8	0.8	35.7
4	3.5	0.0	3.5	15.1	8.0	23.1	0.0	0.0	0.0	26.6
7	0.0	0.0	0.0	13.8	9.5	23.3	0.0	0.0	0.0	23.3

nd – not detected

### III. CONCLUSION

Several samples displayed mass balances <90% AR, however this is considered to be due to losses of <sup>14</sup>CO<sub>2</sub>, which is observed at concentrations of up to 38.9% AR, and therefore does not affect the validity of the study with respect to either the route or rate of degradation of daminozide.

Parent daminozide was completely degraded in both systems by 7 days. Formaldehyde was the only metabolite observed at concentrations > 5% AR, in either the water or sediment phases, reaching maximum concentrations of 17.0% AR, 9.5% AR and 24.1% AR in water, sediment and total system respectively. Though in general peak concentrations were increasing after 7 days, because of the pattern of radioactivity in the aqueous phase and extracted sediments, only small increases of formaldehyde concentrations would be possible in the maximum



sediment and whole system concentrations of formaldehyde, and aqueous phase concentrations would be lower than observed at 7 days.

The study demonstrates that in natural water/sediment systems daminozide is likely to degrade rapidly to formaldehyde which in turn is degraded to CO<sub>2</sub>.

**RMS comments and conclusion:**

Total recoveries were very low and dropped below 90% after 2 days in both water/sediment systems. This can be caused due to losses of <sup>14</sup>CO<sub>2</sub> but there could be losses of methanol as well because it is highly volatile metabolite. Analysis for metabolites in the sediment extract stopped after 7 days. However, the amount of formaldehyde (later identified as methanol, see below) in the sediment extract increased up till that moment. However, due to the fact that no parent is present in the sediment an increase of metabolite is unlikely after 7 days..

Both water systems have a redox potential ≤200 mV, this indicates anoxic conditions.

**Statement submitted by the Notifier**

*The only metabolite observed at concentrations > 5% AR in either the water or sediment phases was reported to be formaldehyde, which reached maximum concentrations of 17.0% AR, 9.5% AR and 24.1% AR in water, sediment and total system respectively. The terminal degradation product was shown to be <sup>14</sup>CO<sub>2</sub> which was present up to at least 38.9% AR 7 days after application. The lower mass balance is due to losses of activity and most probably as CO<sub>2</sub>.*

*Re-examination of the study report of De Vette and van Es, 2002, demonstrates that the study only utilised HPLC analysis with radio-detection, similar to the soil degradation study (Yu and Kobryn, 1993). No co-chromatography was conducted. No confirmatory analytical method was reported and the only reference standard for potential polar metabolites investigated was that formaldehyde.*

*The presented chromatograms, however, show that the same metabolic pattern as observed in Möndel, 2015 soil degradation study was observed. As stated above, a similar HPLC analytical method was used. The analysed <sup>14</sup>C-formaldehyde standard shows again slightly different retention time and a much broader peak when compared to the polar water metabolite. Based on the similarity of the metabolic pattern from Möndel, 2015, it can therefore clearly be assumed that the peak at approximately 7 min is methanol and the peaks at ca. 11 and 26 min are daminozide. The polar metabolite was instantaneously formed in water in the same way as in soil and therefore it can only be methanol.*

**RMS comments:**

Simply comparing peaks it was not clearly demonstrated that unknown metabolite is methanol. Specific analytical method for identification of unknown metabolite should be performed.

<b>Reference:</b>	<b>Hilton, M., Callow, B. (2016)</b> Determination of rates of decline for daminozide and its metabolites in aqueous photolysis and water/sediment studies according to the FOCUS Kinetics Guidance Document
Report No.:	2014-179
Document No:	1007582.UK0-7393
Guideline:	FOCUS Kinetics Guidance Document (FOCUS 2006)
GLP:	No – not applicable
<b>Previous evaluation:</b>	Submitted for the purpose of renewal

## Summary

The degradation of daminozide in irradiated sterile pure water and irradiated sterile natural water was kinetically evaluated according to the recommendations of the FOCUS Kinetics Guidance document. In addition the degradation of daminozide and its polar metabolite were kinetically evaluated in two laboratory water/sediment systems.

The SFO kinetic model provided a very good description of the degradation of daminozide in all systems. In all cases the  $\chi^2$  % error was significantly < 15%, and visual fits and plots of the residuals confirmed the very good fits. P values for the SFO rate constant were generally < 0.05, with the exception of the irradiated sterilised pure water; in this case daminozide degraded only very slowly with DT<sub>50</sub> and DT<sub>90</sub> values extrapolated significantly beyond the study duration.

The irradiated SFO laboratory natural water DT<sub>50</sub> value at 20°C was 36.8 days. Though specific values are uncertain, it is clear that daminozide was degraded very slowly, or is essentially stable to direct photolytic degradation in irradiated pure water systems. Kinetic fitting was not performed for the dark controls in either natural or pure water due to the small number of data points. Nevertheless, comparison of the residues present at the study initiation and termination demonstrates that daminozide was stable in the dark in both systems.

The whole system water/ sediment study DT<sub>50</sub> values for daminozide at 20°C were 0.878 days and 0.935 days, and DT<sub>90</sub> values were 2.92 days and 3.10 days, demonstrating daminozide's very rapid degradation in such systems.

The  $\chi^2$  % error values and visual fits for the SFO kinetic fits of the polar metabolite indicated that SFO kinetics provided an acceptable description of the degradation. Both fits provided t-test statistics > 0.1, which are likely to be due to the lack of a decline phase, the large, but random, scatter of measured polar metabolite residues, and extrapolation of the DT<sub>50</sub> and DT<sub>90</sub> values beyond the study duration. Overall, both fits were considered acceptable. The calculated whole system DT<sub>50</sub> values at 20°C for the polar metabolite were 37.8 days and 93.4 days, with formation fractions of 0.283 and 0.258.

The purpose of this study is to re-calculate the rates of degradation of daminozide in the aqueous photolysis study of Brice and Scholey (2006) and the water/ sediment study of De Vette and van Es (2002). Re-calculations of degradation rates have been performed according to the guidance within the FOCUS Degradation Kinetics Report.

## Materials and Methods

### Details of the studies

In Brice and Scholey (2006) the aqueous photolytic degradation of daminozide was investigated in both pure water and natural stream water. Sterilised waters were treated with [1,4-<sup>14</sup>C]-daminozide at a nominal application rate

of 2.5 mg/kg, and the samples were continuously irradiated with a xenon lamp filtered to remove light  $< 290$  nm for a maximum of 15 days and at  $25 \pm 2^\circ\text{C}$ . Dark control samples of both the natural and pure water were treated at the same rate and incubated. Single samples were collected at various time-points and the detections of  $^{14}\text{C}$ -daminozide are given in Tables 1 and 2 alongside values to be used in the kinetic evaluation, following treatment of the raw data in accordance with FOCUS Kinetics guidance.

In De Vette and van Es (2002) two water/ sediment systems (TNO ditch and Kromme Rijn river) were treated with  $^{14}\text{C}$ -daminozide at a nominal application rate of 1 mg/ L. Test systems were incubated in the dark at approximately  $20^\circ\text{C}$  for up to 21 days. The metabolite formaldehyde was reported to have been observed at concentrations  $> 10$  % AR in the whole water/ sediment system. However, re-examination of the study and the analytical method used for the identification of the polar metabolite as formaldehyde demonstrates that the metabolite may also have been other polar metabolites. The detections of  $^{14}\text{C}$ -daminozide and its polar metabolite in the individual water and sediment phases and in the whole system are given in Tables 3 and 4. The total system residues are used for the kinetic evaluations, following treatment according to FOCUS Kinetics guidance.

The modelling was performed using KINGUII version 2 (Bayer Crop Science 2011).

The degradation scheme assumed for samples in the water/ sediment study is presented in Figure B.8.2.2.3-1.

Two levels of kinetics are proposed for the determination of rates of degradation from sediment/water studies. P-I is for single compartment approaches and P-II is proposed for multi-compartment approaches where degradation in both the water and sediment is considered. In this exercise only the single compartment (P-I) approach was used for the kinetic evaluation of degradation of daminozide in the whole system. For the determination of metabolite degradation kinetics the term M-I is used to describe single compartment approaches. The M-I approach was used for the determination of metabolite degradation in the whole system.

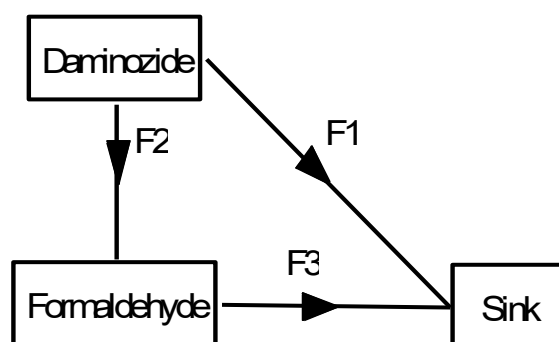


Figure B.8.2.2.3-1: Degradation scheme used in kinetic evaluation of the water/ sediment study of De Vette and van Es (2002)

Table B.8.2.2.3-6: Summary of the results of the kinetic determinations for daminozide in irradiated sterilised pure and natural water in Brice and Scholey 2006

Model	Parameter	Irradiated Sterilised Pure Water	Irradiated Sterilised Natural Water
SFO	Visual Fit	Good	Very good
	$\chi^2$ error (%)	1.11	2.10
	P	0.161	0.0002
	Lower CI	-0.000999	0.0144
	Upper CI	0.004	0.023
	k	0.0013	0.0188
	DT <sub>50</sub>	546	36.8
FOMC	DT <sub>90</sub>	1813	122
	Visual Fit	Good	Very good
	$\chi^2$ error (%)	0.90	2.25
	$\alpha^*$	2.34E-3 ± NC	0.935 ± 7.896
	$\beta^*$	7.57E-6 ± NC	42.91 ± 416.2
	DT <sub>50</sub>	3.57E123	47.2
	DT <sub>90</sub>	-	460.8

\* Confidence intervals are also reported, since t-test statistics are not appropriate determinants of the certainty of the parameters  $\alpha$  and  $\beta$ .

NC – Not calculated.

Fits presented in bold font are considered the most appropriate to derive both persistence and modelling end-points.

Table B.8.2.2.3-7: Summary of the results of the kinetic determinations for daminozide and the polar metabolite in the water/ sediment study of De Vette and van Es 2002 (whole system)

Model	Parameter	TNO Ditch		Kromme Rijn Stream	
		Daminozide	Polar Metabolite	Daminozide	Polar Metabolite
SFO	Visual Fit	Good	Very good	Very good	Excellent
	$\chi^2$ error (%)	10.6	8.30	5.72	1.89
	P	4.87E-9	0.229	1.27E-10	0.428
	k	0.742	0.0184	0.790	0.0074
	Lower CI	0.586	-0.029	0.661	-0.071
	Upper CI	0.897	0.066	0.919	0.086
	DT <sub>50</sub>	0.935	37.8	0.878	93.4
	DT <sub>90</sub>	3.10	126	2.92	310
	FF	-	0.283	-	0.258
FOMC	Visual Fit	Good	-	Very good	-
	$\chi^2$ error (%)	9.18	-	5.72	-
	$\alpha^*$	2.87 ± 3.65	-	4.5E+3 ± 192916	-
	$\beta^*$	3.17 ± 5.02	-	5.7E+3 ± 244214	-
	DT <sub>50</sub>	0.867	-	0.877	-
	DT <sub>90</sub>	3.91	-	2.92	-

\* Confidence intervals are also reported, since t-test statistics are not appropriate determinants of the certainty of the parameters  $\alpha$  and  $\beta$ .

NC – Not calculated

Fits presented in bold font are considered the most appropriate to derive both persistence and modelling end-points.

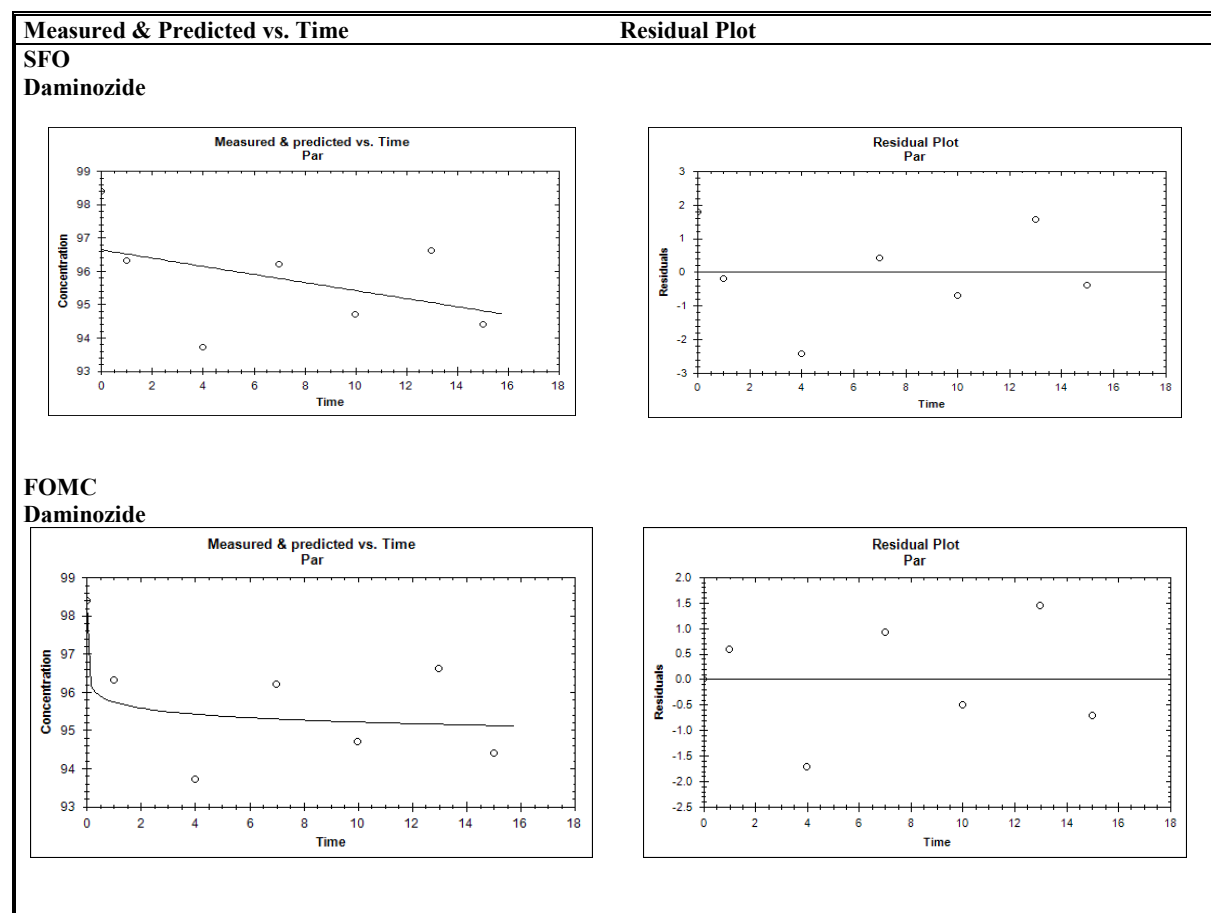


Figure B.8.2.2.3-2: Plots of the decline and the residuals from KINGUII for daminozide in an irradiated sterilised pure water – SFO

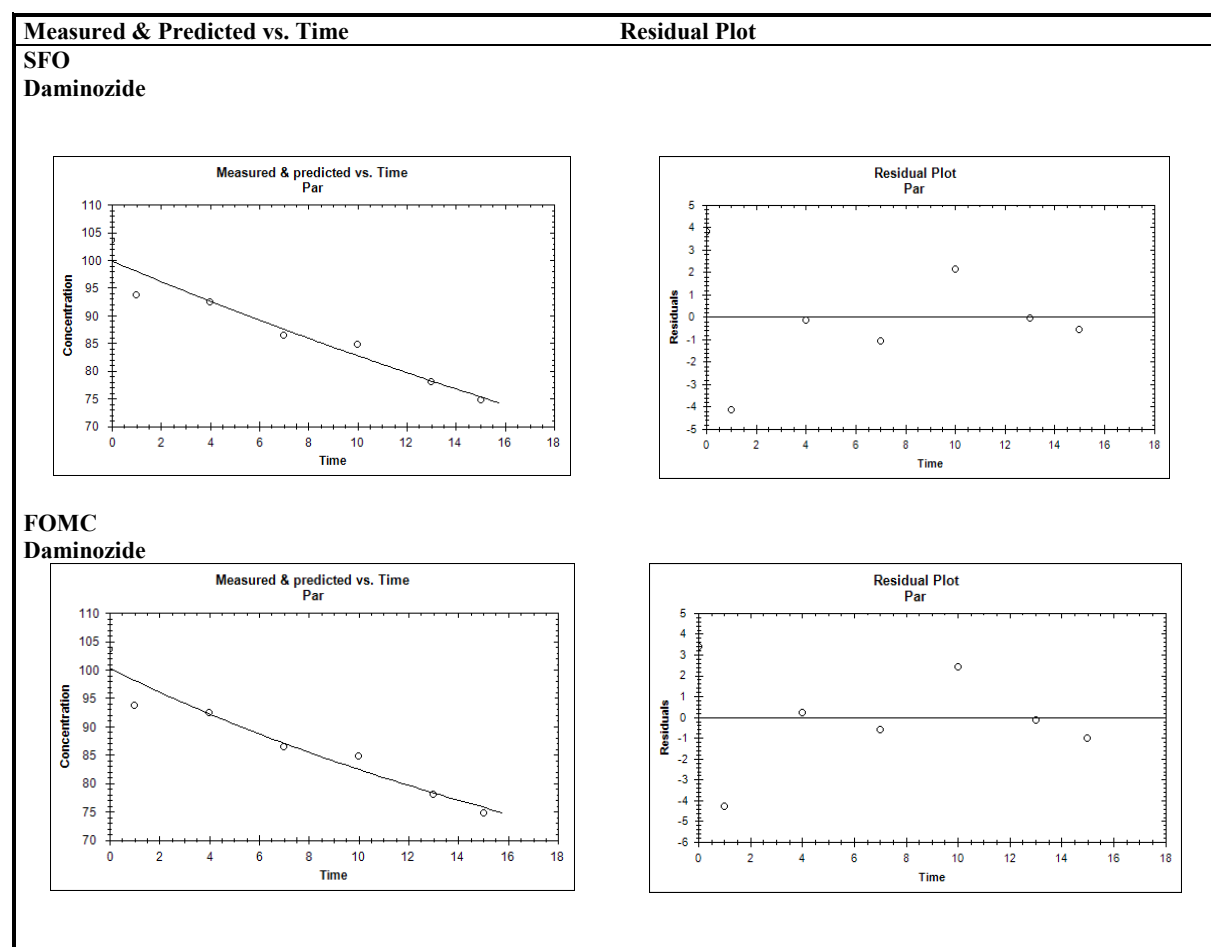


Figure B.8.2.2.3-3: Plots of the decline and the residuals from KINGUII for daminozide in an irradiated sterilised natural water - SFO

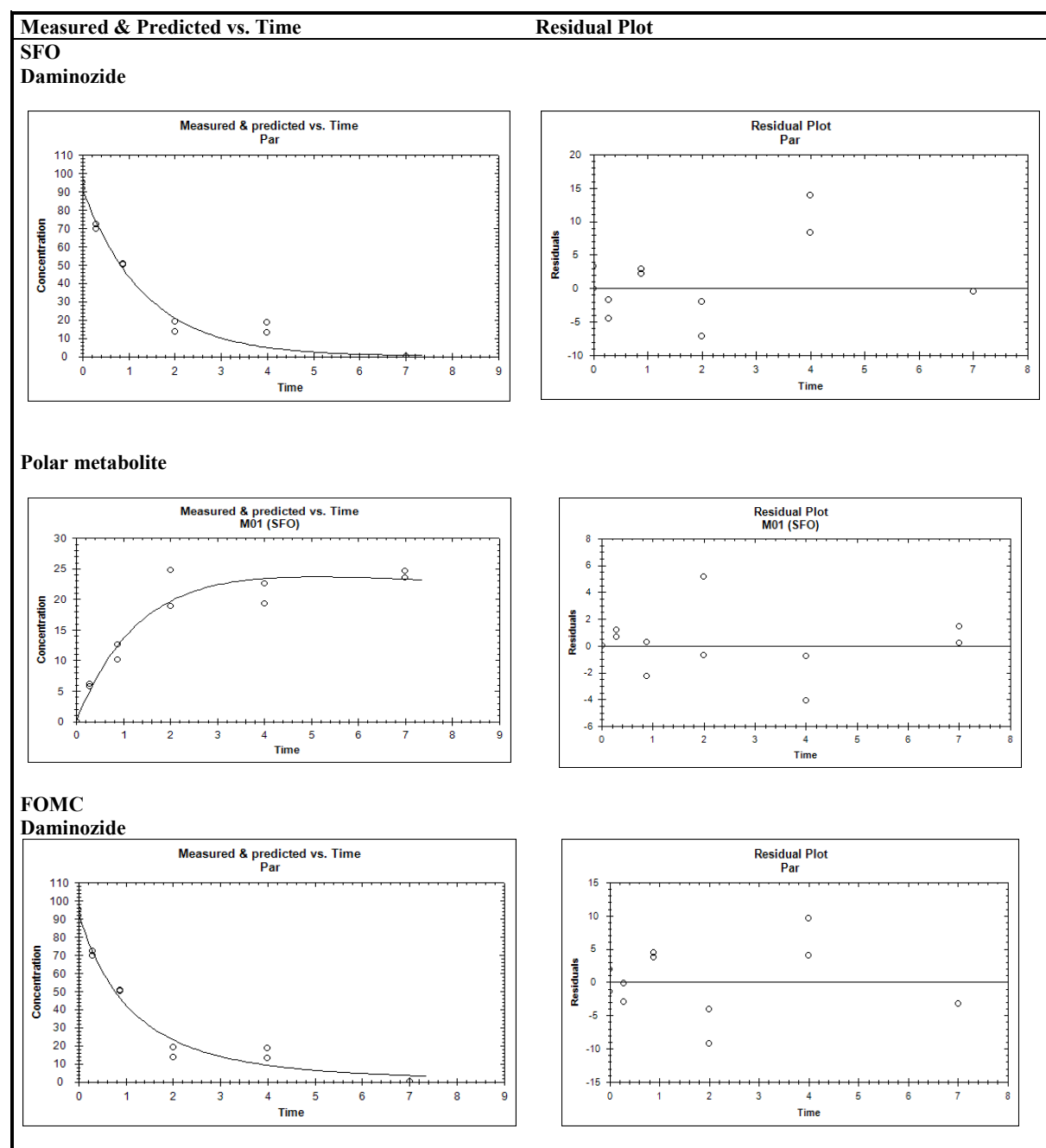


Figure B.8.2.2.3-4: Plots of the decline and the residuals from KINGUII for daminozide and its polar metabolite in the TNO ditch whole water/ sediment system

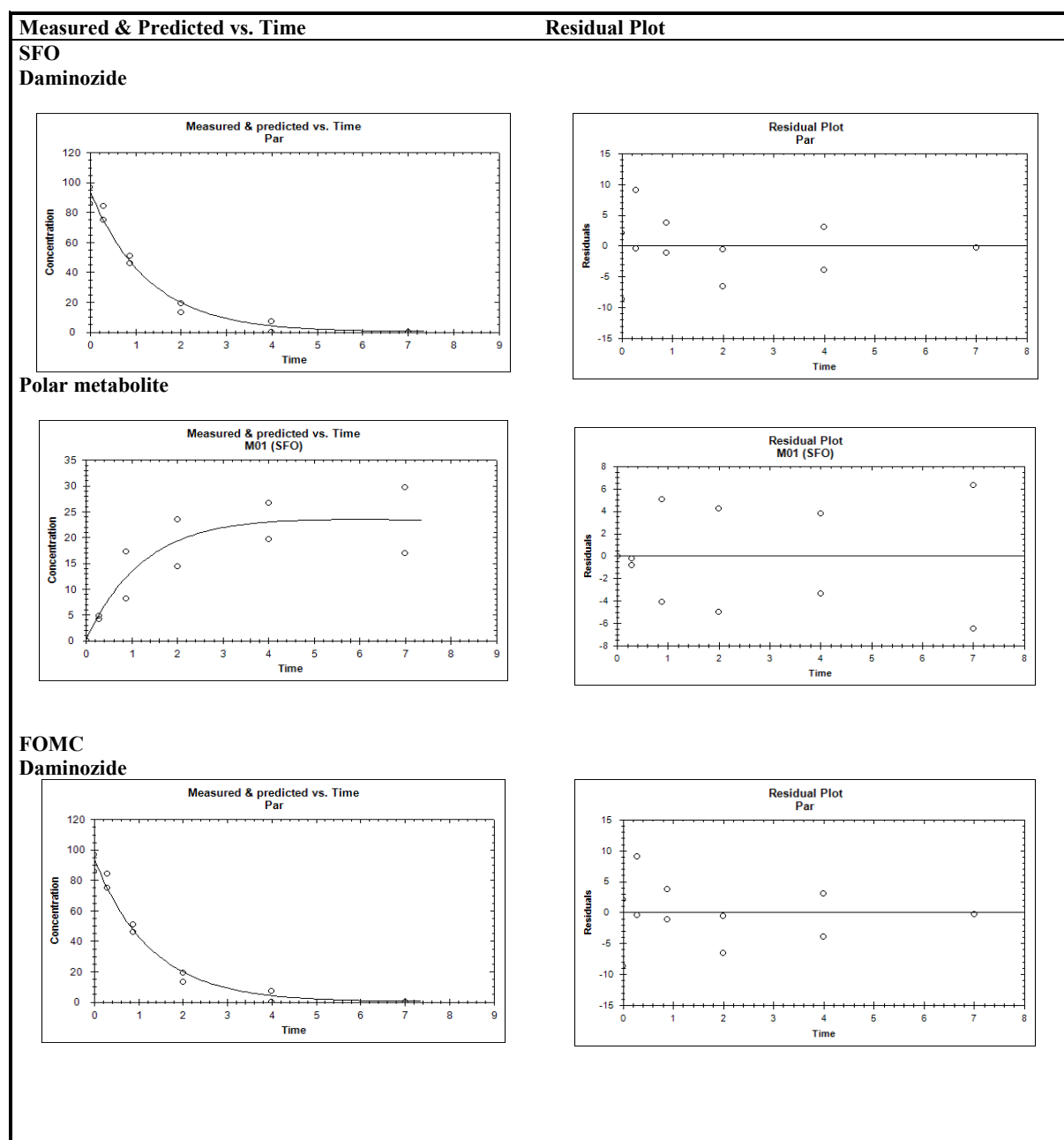


Figure B.8.2.2.3-5: Plots of the decline and the residuals from KINGUII for daminozide and its polar metabolite in the Kromme Rijn whole water/ sediment system - SFO

## Conclusion

Examination of kinetic fitting statistics and visual fits demonstrates that the SFO kinetic model provided a very good description of the degradation of daminozide in all systems studied.

The SFO irradiated natural water  $DT_{50}$  value for daminozide at 20°C was 36.8 days. Though specific values are uncertain, it is clear that daminozide was degraded only very slowly, or is essentially stable to direct photolytic degradation in irradiated pure water systems. Comparison of the residues present at the study initiation and termination demonstrates that daminozide was stable in the dark in both natural and pure water systems.



The SFO whole system water/ sediment study DT<sub>50</sub> values for daminozide at 20°C were 0.878 days and 0.935 days, and DT<sub>90</sub> values were 2.92 days and 3.10 days, demonstrating daminozide's very rapid degradation in such systems.

The visual fits and statistical parameters for the SFO kinetic fits of the degradation of the metabolite in water/ sediment systems indicated that SFO kinetics provided an acceptable description of the degradation. The calculated whole system DT<sub>50</sub> values at 20°C for the polar metabolite were 37.8 days and 93.4 days, with formation fractions of 0.283 and 0.258.

#### **RMS comments and conclusion:**

Photodegradation – SFO and FOMC kinetics fit for daminozide in sterilised pure water is not statistically and visually acceptable. Daminozide is stable in the sterilised pure water. The SFO kinetics for daminozide in sterilised natural water gave visually and statistically acceptable fit, both parameters  $\alpha$  and  $\beta$  for FOMC kinetics contain zero. Therefore, SFO kinetics for natural water is accepted by the RMS.

Water/sediment study - SFO kinetic analyses for parent compound daminozide is visually and statistically acceptable. Whole system SFO DT<sub>50</sub> and DT<sub>90</sub> values for polar metabolite are extrapolated beyond the study duration and the maximum formation of the metabolite has not been reached during the 7 days of analyses. SFO kinetics is statistically not acceptable as confidence intervals of the rate constant contains zero and also failed t-test. In conclusion, DT<sub>50</sub> and DT<sub>90</sub> values for the metabolite are not accepted by the RMS.

### B.8.2.3 Degradation in the saturated zone

No data were evaluated during the first EU review, and none were required. In accordance Comm. Reg. (EU) No. 283/2013 which sets out the active substance data requirements in accordance with Comm. Reg (EC) No. 1107/2009, no additional data are submitted and none are required.

## B.8.3 Fate and behaviour in air

### B.8.3.1 Route and rate of degradation in air

The vapour pressure and Henry's Law Constant for daminozide are  $1.5 \times 10^{-6}$  Pa at 25°C and  $1 \times 10^{-9}$  Pa m<sup>3</sup>/mole, indicating the low volatility of daminozide.

The Atkinson half-life of daminozide was calculated using AOPWIN v.1.92, assuming a 12-hour day and a hydroxyl radical concentration of  $1.5 \times 10^6$  cm<sup>-3</sup>. A half-life in the upper atmosphere of 10.570 hours or 0.881 days (based on a 12 hour day) was calculated.

Considering all of the above daminozide is not anticipated to be volatilised to air. Any daminozide that is volatilised would be anticipated to be rapidly degraded. Daminozide is not anticipated to be subject to long range transport.

Methanol is known to be volatile, and a vapour pressure of  $1.69 \times 10^4$  Pa at 25°C and a Henry's Law constant of 0.46 Pa.m<sup>3</sup>/mole at 25°C were obtained using the EPIWEB 4.1 experimental database. The Atkinson half-life of methanol was calculated in the same manner as for daminozide, as 17.36 days (based on a 12 hour day).

### B.8.3.2 Transport via air

No data have been submitted by the Notifier.

### RMS comments and conclusion:

Metabolite methanol is a subject to short range and long range transport, see separate Annex B.8 for the product data (B.8.6.2).

### B.8.3.3 Local and global effects

The data requirements stipulated in Comm. Reg. (EU) No. 283/2013 which sets out the active substance data requirements in accordance with Comm. Reg (EC) No. 1107/2009, require that for substances that are applied in high amounts, atmospheric effects are required to be considered should it not be possible to rule out long-range transport on the basis of a substances volatility and Atkinson half-life. However, methanol is not applied in high amounts and therefore further investigation is not required. The proposed use of daminozide is for a maximum application of 5 x 7.65 kg a.s./ha indoors or 5 x 4.25 kg a.s./ha in the field and methanol is observed at a maximum of 27% AR in aerobic soil degradation studies. The amount of methanol formed from the use of daminozide is

insignificant when compared to the tonnage of methanol registered under REACH (Regulation (EC) No. 1907/2006) of 10,000,000 – 100,000,000 tonnes of methanol used per annum in the EU. Numerous uses have been assessed under the REACH regulation including several outdoor uses with environmental release directly to air. No exposure controls are in place for those uses and the exposure of methanol to air from the proposed use of daminozide is anticipated to be significantly lower than uses approved under the REACH regulation. Therefore, further consideration of the local and global effects of methanol is not required and no additional data are submitted.

**RMS comments and conclusion:**

The total amount of daminozide used in the EU per annum should be presented as confidential information in Section CP.1.4.1 of Doc. J of this dossier. But the information is not clearly stated there (only the amount of manufactured product is available). Application rate for field use is relatively high and formation of the methanol in soil is 27% and in surface water up to 75.7 % AR. Therefore, the amount of daminozide used in the EU should be presented by the Notifier and based on data local and global effects should probably be addressed.

**Notifier's statement:**

The total amount of daminozide used in the EU can be found in the updated Documents J for the daminozide Task Force members which have been amended as requested. This tonnage compares to the 10,000,000-100,000,000 tonnes of methanol registered in the EU under the REACH regulation. It should also be noted that methanol is a substance that is naturally produced in large amounts. Additionally, the following is concluded in Section 7.8 of the FOCUS AIR (SANCO/10553/2006 [2008]) guidance document:

*“emissions of currently used pesticides are likely to be negligible compared of emissions of other substances such as CO<sub>2</sub>, and hence the effects on the atmosphere are likely to be marginal in comparison. This point needs to be born in mind when considering the potential effects of pesticides on the atmosphere.”*

In the conclusion of the FOCUS Air report it is also stated that for substances that are applied in high volumes the following adverse effects can potentially occur:

- Global Warming Potential (GWP) – only if the chemical is volatile, has a strong IR absorption (800-1200 cm<sup>-1</sup>) and long residence time (> 1 year)
- Ozone depletion potential (ODP) in the stratosphere, only if the chemical is volatile and atmospheric residence time of >1 year. In the same report it is noted that only a chemical that contains one or more Cl or Br substituents has a potential to impact on stratospheric ozone. “Other halogens do not play an important role in the stratospheric depletion”.
- Accumulation in the troposphere, only if the chemical is a gas and the atmospheric residence time is >20 years”

Methanol is known to be volatile with a vapour pressure of  $1.69 \times 10^4$  Pa at 25°C and a Henry's Law Constant of 0.46 Pa.m<sup>3</sup>/mole at 25°C obtained using the EPIWEB 4.1 experimental database. The Atkinson half-life of methanol was calculated as 17.36 days (based on a 12 hour day) and therefore none of the above effects are likely

for methanol. In addition methanol does not contain either Cl or Br, further evidence that it has a low ozone depletion potential. It also contains none of the atoms (Cl, F, N or S) likely to be responsible for acidic compounds nor any of the atoms (P or N) responsible for eutrophication. Therefore, its acidification and eutrophication potential are also very low. Therefore, the long transport of methanol and any subsequent potential local and global effects are not considered to be of any concern.

**RMS 's conclusion:** agrees with the Notifier that local and global effects of methanol are expected to be negligible.

#### **B.8.4 Monitoring data concerning fate and behaviour of the active substance, metabolites, degradation and reaction products**

No data were evaluated during the first EU review, and none were required. In accordance Comm. Reg. (EU) No. 283/2013 which sets out the active substance data requirements in accordance with Comm. Reg (EC) No. 1107/2009, monitoring data are required to be submitted where they are available. No monitoring data are available for daminozide and therefore none are submitted and none are required.

#### **B.8.5 References relied on**

##### **Literature search**

A literature search was carried out by the notifier in accordance with Article 7, Paragraph 1(m) of Commission Implementing Regulation (EU) No. 844/2012.

The search was performed for active substance daminozide and daminozide metabolites and relevant impurities:

- Daminozide (common name)
- 1596-84-5 (CAS Number)
- UDMH (unsymmetrical dimethyl hydrazine)
- 57-14-7 (CAS Number)
- NDMA (N,N-dimethylnitrosamine)
- 62-75-9 (CAS Number)
- Methanol
- 67-56-1 (CAS number)
- 200-659-6 (EC number)

A series of searches were carried out using the STN and the Dialog platforms:

- an initial search covering studies published from 2004 to 2011;
- additional searches to cover the time periods 2012 to 2013, 2013 to 2014 and February to December 2014;
- a separate search for all in vitro/in vivo metabolism studies excluding hits preceding 2004 (to address the requirement under Regulation (EU) No. 283/2013 for comparative in vitro metabolism studies);
- a separate search covering the metabolite methanol from 2005 to 2015.

These literature searches were performed to cover the 10 years prior to the expected submission of the AIR 3 dossier for daminozide which was submitted for review in April 2015.

**Table B.8.5-1: Summary of the literature review**

<b>Summary of the review</b>	<b>n</b>
Total number of summary records retrieved after removing duplicates from all database searches	2134
Number of summary records excluded after rapid assessment for relevance (by title/abstract)	2074
Number of summary records of potential/unclear relevance assessed in further detail (by abstract/full-text)	60
Number of studies excluded from further consideration after detailed assessment for relevance (by abstract/full-text)	48
Number of studies not excluded for relevance after detailed assessment (i.e. relevant studies and studies of unclear relevance)	12
Number of relevant and reliable studies (Klimisch criteria 1-2) identified by the literature search and appraisal process	8
Number of studies considered relevant and reliable relate to EFATE	0

As part of the determination of relevancy, the following criteria are considered to be fundamental when considering the relevance of an open-literature study:

- Generally, degradation studies are considered relevant if they are carried out with the active substance only, and not with mixtures, since this may significantly influence the degradation behaviour. For laboratory soil degradation studies, the substrate used needs to be considered; in order to realistically reflect agro-ecosystems, it is crucial that the study is conducted with soil and that the soil is not contaminated and is representative of European agricultural soils. Temperature and moisture should be considered as reliability criteria. For field studies, relevance is based on (pedo-)climatic conditions being representative for European agriculture.
- The application of the test material needs to be considered because studies are not considered relevant if the application rates are significantly outside the representative use or the active substance is applied as a by-product (e.g. as a component of organic soil amendments).
- For adsorption studies, the substrate used needs to be considered.
- Relevance criteria for the aquatic compartment are analogous to those of soil-related data requirements.
- Monitoring studies, including those for air, may be considered relevant if the areas investigated are representative for Europe. Studies which are purely analytical, i.e. they determine levels of the active substance in certain environmental compartments, are not considered as relevant

The criteria considered for relevancy of studies relating to individual environmental fate data requirements are detailed in the table below:

**Table B.8.5-2: Relevancy criteria considered**

<b>Data requirement (data point)</b>	<b>Relevancy criteria considered</b>
<b>Active substance</b>	

Fate and behaviour in soil (KCA 7.1)	<ol style="list-style-type: none"> <li>1. Well-defined test material applied as active substance or plant protection product (not as a by-product or ingredient of a soil amendment).</li> <li>2. Substrate is a representative soil for agricultural uses with well-defined soil properties (e.g. pH, organic carbon content, microbial biomass etc). This is also relevant for field studies.</li> <li>3. No previous contamination of the soil.</li> <li>4. Active substance is not applied as a mixture with other active substances.</li> </ol>
Fate and behaviour in water and sediment (KCA 7.2)	<ol style="list-style-type: none"> <li>1. Well-defined test material applied as active substance or plant protection product.</li> <li>2. Test samples used are samples from representative European aquatic resources with no contamination</li> <li>3. Active substance is not applied as a mixture with other active substances.</li> </ol>
Fate and behaviour in air (KCA 7.3)	<ol style="list-style-type: none"> <li>1. Well-defined test material.</li> <li>2. Areas investigated are relevant for Europe.</li> </ol>

#### RMS comments and conclusion:

A literature search was performed according to EFSA guidance on submission of scientific literature (EFSA Journal 2011;9(2):2092. The RMS agrees with the the literature search performed by the Notifier. No relevant and reliable study has been found in the literature search.

#### 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
CA 7.1.1.1 /01	Möndel, M.,	2015	Degradation of [ <sup>14</sup> C]-Daminozide in four soils incubated under aerobic conditions at 20 ± 2 °C in the dark Agroscience. Report No. AS358 GLP Unpublished Previous evaluation: Submitted for the purpose of renewal.	N	Y	New data for AIR3 renewal	EU Daminozide Task Force
CA 7.1.1.1 /02	Jones, M.,	2015	Daminozide: Analysis of Unknown Metabolites in Soil Selcia. Report No. SEL/8273/1 Non-GLP Unpublished Previous evaluation: Submitted for the purpose of renewal.	N	Y	New data for AIR3 renewal	EU Daminozide Task Force
CA 7.1.1.1 /03	DeMaio, W.	2015	Degradation of [ <sup>14</sup> C]-Daminozide in Four Soils Incubated Under Aerobic Conditions at 20 ± 2 °C in the Dark: Characterization of a Daminozide Degradation Product Report No. 033709-1 Non-GLP Unpublished Previous evaluation: Submitted for the purpose of renewal.	N	Y	New data for AIR3 renewal	EU Daminozide Task Force
CA 7.2.1.2 /01	Brice, A., Scholey, A.	2006	[ <sup>14</sup> C]-Daminozide: photodegradation in sterile, aqueous solution Covance Laboratories Ltd. Report No. 2242/044 GLP Unpublished Previous evaluation: Submitted for the purpose of renewal.	N	Y	New data for AIR3 renewal	Fine Agrochemicals Limited
CA 7.2.1.2 /02	Hilton, M., Callow, B.	2016	Determination of rates of decline for daminozide and its metabolites in aqueous photolysis and water/sediment studies according to FOCUS kinetics guidance document Document No. 1007582.UK0/7393 Non-GLP Unpublished Previous evaluation: Submitted for the purpose of renewal.	N	N	Not applicable	EU Daminozide Task Force
CA 7.2.2.2 /01	Button, S.	2015	Daminozide: Aerobic mineralisation in surface water (pelagic test) Huntingdon Life Sciences. Report No. FDD0108 GLP Unpublished Previous evaluation: Submitted for the purpose of renewal.	N	Y	Not applicable	EU Daminozide Task Force

**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
CA 7.1.1.1.1/01	Dannals, L., Puhl, R., Kucharczyk, N.	1972	Environmental Fate Studies on Alar: Second Status Report on PR 70-15 Generated by: (Not identified in report) Submitted by: Uniroyal Chemical Uniroyal file No. A.8.1.3 Date: March 27, 1972 Not GLP, Unpublished Previous evaluation: In DAR 1999	N	N		Arysta LifeScience Great Britain Limited
CA 7.1.1.1.1/02	Dannals, L., Puhl, R., Kucharczyk, N.	1994	Dissipation and Degradation of Alar® in Soils Under Greenhouse Conditions Generated by: Uniroyal Chemical Submitted by: Fine Agrochemicals Ltd. FAL file No. (not provided) Date: 1974 Not GLP, Published Previous evaluation: In DAR 1999	N	N	Not applicable	Arysta LifeScience Great Britain Limited
CA 7.1.1.1.1/03	Goodyear, A.	1995	(14C)-Daminozide Soil metabolism and Degradation Generated by: Corning Hazleton Submitted by: JSC International Ltd. for Fine Agrochemical Ltd. FAL file No. (not provided) (report #1137/2-1015) Date: August 1995 GLP, Unpublished Previous evaluation: In DAR 1999	N	N	Not applicable	FAL
CA 7.1.1.1.1/04	Yu, W.	1993	Characterization of Volatiles from Daminozide Aerobic Soil Metabolism Generated by: Uniroyal Chemical Submitted by: Uniroyal Chemical Uniroyal file No. not provided Date: November 4, 1993 GLP, Unpublished Previous evaluation: In DAR 1999	N	N	Not applicable	Arysta LifeScience Great Britain Limited
CA 7.1.1.1.1/05	Yu, W., Kobryn, K.	1993	Daminozide Aerobic Soil Metabolism Generated by: Uniroyal Chemical Submitted by: Uniroyal Chemical Uniroyal file No. A.8.1.21 Date: March 3, 1993 GLP, Unpublished Previous evaluation: In DAR 1999	N	N	Not applicable	Arysta LifeScience Great Britain Limited
CA 7.1.1.2/01	Dzialo, D.G., Harned, W.H.	1986	Daminozide aerobic and anaerobic soil metabolism studies Uniroyal Chemical. Report No. A.8.1.5 Non-GLP Unpublished Previous evaluation: In DAR 1999	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
CA 7.1.1.1.2/01	Lengen, M. Dzialo, D.	1984	Daminozide Photolysis in Water and on Soil (Interim Report) Generated by: Uniroyal Chemical Submitted by: Uniroyal Chemical Uniroyal file No. A.8.1.6 Date: December 1984 Not GLP, Unpublished Previous evaluation: In DAR 1999	N	N	Not applicable	Arysta LifeScience Great Britain Limited
CA 7.1.1.1.2/02	Lengen, M.	1985	Addendum to: Hydrolysis of Daminozide (Project No. 84244) and Daminozide Photolysis in Water and on Soil (Project No. 84246) Generated by: Uniroyal Chemical Submitted by: Uniroyal Chemical Uniroyal file No. A.8.1.9 Date: June 1985 Not GLP, Unpublished Previous evaluation: In DAR 1999	N	N	Not applicable	Arysta LifeScience Great Britain Limited
CA 7.1.3.1.1/01	Beeching, A.	1987	Determination of adsorption isotherms for daminozide on two soil types Resource Consultants Cambridge Ltd. Report No. FAL 0018 Non-GLP Unpublished Previous evaluation: In DAR 1999	N	N	Not applicable	Fine Agrochemicals Ltd.
CA 7.1.3.1.1/02	Spare, W.	1987	Determination of the adsorption/desorption constants of ALAR Agriseach Incorporated. Report No. A.8.1.11 Non-GLP Unpublished Previous evaluation: In DAR 1999	N	N	Not applicable	Arysta LifeScience Great Britain Limited
CA 7.1.4.1.1/01	McManus, J., Dzialo, D., Lengen, M.	1984	Daminozide column leaching study with aged sandy loam Uniroyal Chemical. Report No. A.8.1.8 Non-GLP Unpublished Previous evaluation: In DAR 1999	N	N	Not applicable	Arysta LifeScience Great Britain Limited
CA 7.2.1.1/01	Lengen, M.	1982	Hydrolysis of Daminozide Generated by: Uniroyal Chemical Submitted by: Uniroyal Chemical Uniroyal file No. A.8.1.12 Date: February 1982 Not GLP, Unpublished Previous evaluation: In DAR 1999	N	N	Not applicable	Arysta LifeScience Great Britain Limited
CA 7.2.1.1/02	Lengen, M., Abdel-Kader, H., Peterson, G.	1984	Hydrolysis of Daminozide Generated by: Uniroyal Chemical Submitted by: Uniroyal Chemical Uniroyal file No. A.8.1.4 Date: December 1984 Not GLP, Unpublished Previous evaluation: In DAR 1999	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
CA 7.2.2.1 /01	Ritter, A.	1989 a	Ready Biodegradability, Modified OECD Screening Test for Alar Technical RCC Umweltchemie AG. Report No. A.8.1.19 GLP Unpublished Previous evaluation: In DAR 1999	N	N	Not applicable	Arysta LifeScience Great Britain Limited
CA 7.2.2.3 /01	De Vette, H.Q.M., van Es, C.	2002	A study on the degradation of [ <sup>14</sup> C] daminozide in two water/sediment systems TNO Chemistry. Report No. 01-2482/01 GLP Unpublished Previous evaluation: In Addendum II (2002)	N	N	Not applicable	Arysta LifeScience Great Britain Limited