Committee for Risk Assessment
RAC

Annex 1
Background document
to the Opinion proposing harmonised classification and labelling at EU level of

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

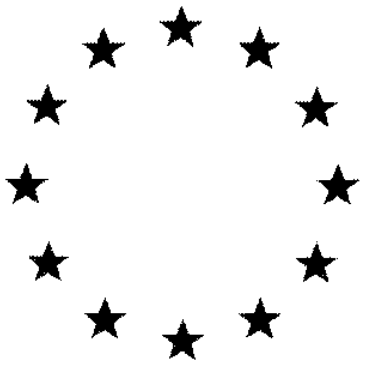
EC Number: 216-485-9
CAS Number: 1596-84-5

CLH-O-0000006804-70-01/F

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It includes the proposal of the dossier submitter and the conclusion of RAC. It is based on the official CLH report submitted to public consultation. RAC has not changed the text of this CLH report but inserted text which is specifically marked as 'RAC evaluation'. Only the RAC text reflects the view of RAC.

Adopted
11 June 2020

European Commission



Draft (Renewal) Assessment Report prepared according to the Commission Regulation (EC) No 1107/2009
and
Proposal for Harmonised Classification and Labelling (CLH Report) according to Regulation (EC) N° 1272/2008

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

Volume 1

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

ANNEX 1 – BACKGROUND DOCUMENT TO RAC OPINION ON DAMINOZIDE (ISO); 4-(2,2-DIMETHYLHYDRAZINO)-4-OXOBUTANOIC ACID; N-DIMETHYLAMINOSUCCINAMIC ACID

Version history page

Date	Version	Reason for revision
May 2018	Version 1	First draft
October 2018	Version 2	Notifier's and co-RMS comments
January 2019	Version 3	Update to include CLH Report
June 2019	Version 4	Update following the ECHA accordance check

ANNEX 1 – BACKGROUND DOCUMENT TO RAC OPINION ON DAMINOZIDE (ISO); 4-(2,2-DIMETHYLHYDRAZINO)-4-OXOBUTANOIC ACID; N-DIMETHYLAMINOSUCCINAMIC ACID

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1 STATEMENT OF SUBJECT MATTER AND PURPOSE FOR WHICH THIS REPORT HAS BEEN PREPARED AND BACKGROUND INFORMATION ON THE APPLICATION

1.1 Context in which the renewal assessment report was prepared

1.1.1 Purpose for which the renewal assessment report was prepared

This renewal assessment report (RAR) has been prepared according to Regulation (EC) No 1107/2009 of the European parliament and of the council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC and Commission Implementing Regulation (EU) 844/2012 of 18 September 2012 setting out the provisions necessary for the implementation of the renewal procedure for active substances, as provided for in Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market.

Daminozide (ISO); 4-(2,2-dimethylhydrazino)-4-oxobutanoic acid; *N*-dimethylaminosuccinamic acid ('hereafter referred to as 'daminozide') was the existing active substance to be included in Annex I of Council Directive 91/414/EEC by Commission Directive 2005/53/EC of 16 September 2005. The active substance was subsequently deemed to be approved under Regulation (EC) 1107/2009 via Implementing Regulation (EU) 540/2011 of 25 May 2011. The approval expires on 30 October 2018.

The present submission for Annex I Renewal (AIR3 program) is carried out in accordance to the Regulation EC/844/2012 and guidance document SANCO/2012/11251 rev. 1.2 – on the renewal of active substances included in Annex I of Drective 91/414. This Renewal Assessment Report (RAR) has been produced as a result of evaluation of the dossier submitted by EU Daminozide Task Force, consisting of Fine Agrochemicals Limited and Arysta LifeScience Great Britain Ltd (wholly owned subsidiary of Arysta LifeScience Inc., formerly known as MacDermid Agricultural Solutions Inc., and before that as Chemtura Corporation), as main applicant and owner of a complete data package in order to support the renewal of approval of daminozide according to Regulation (EC) No. 1107/2009.

1.1.2 Arrangements between Rapporteur Member State and Co-Rapporteur Member State

In compliance with Commision Implementing Regulation (EU) No. 686/2012 the Czech Republic was appointed as the Rapporteur Member State and Hungary as the Co-Rapporteur Member State for the active substance daminozide. The Co-RMS conducted a peer-review of the RAR.

1.1.3 EU Regulatory history for use in Plant Protection Products

Daminozide was included in Annex I to Directive 91/414/EEC and an existing active substance (EAS) by Commission Directive 2005/53/EC of 16 September 2005. Inclusion entered into force on 1 March 2006. The Rapporteur Member State was the Netherlands. Uniroyal Chemical (Crompton Europe Ltd.) and Fine Agrochemicals Ltd were considered to be the main data submitters. The final Commission Review Report (SANCO3043/99 final) was published on 15 February 2005 and provided endpoints agreed during the first inclusion evaluation (appendix I and II of the Review Report).

Czech Republic and Hungary, being the designated Rapporteur Member State (RMS) and Co-Rapporteur Member State (Co-RMS) respectively, received an application for the renewal of daminozide submitted EU Daminozide Task Force within the 3-years deadline required by Regulation (EU) 844/2012 (art. 1). (Deadline: 28 February 2013, Date of Receipt: 27 February 2013). The completeness of application was confirmed by the RMS in communication to the applicant the co-RMS, the Commission and EFSA on 15 April 2013.

A supplementary dossier carried out in accordance to the Regulation (EU) no. 844/2012 and guidance document SANCO/2012/11251 rev. 1.2 on the renewal of active substances was submitted to the RMS within the deadline of 30 months before expiry of the approval required by Regulation no. 844/2012 (art. 6.3) (Deadline 30 April 2015, Date of Receipt: 30 April 2015). The completeness of the supplementary dossier was confirmed by the RMS in communication to the applicant, co-RMS, the Commission and EFSA on 15 June 2015.

1.1.4 Evaluations carried out under other regulatory contexts

None available.

1.2 Applicant(s) information

1.2.1 Name and address of applicant(s) for approval of the active substance

The original notifiers supporting Daminozide for the first inclusion were Fine Agrochemicals Limited and Uniroyal Chemical Limited. The applicant for the renewal is the EU Daminozide Task Force. The EU Daminozide Task Force is an equal partnership between:

1) **Arysta LifeScience Great Britain Limited** (formerly: MacDermid Agricultural Solutions Incorporated, Chemtura Europe Limited and Uniroyal Chemical Limited)

Registered company address:	3-5 Melville Street Edinburgh EH3 7 PE United Kingdom
Correspondence address:	Brooklands Farm Cheltenham Road Evesham Worcestershire WR11 2LS United Kingdom
Contact:	Mr Graham Evans
Telephone number:	+44 1753 555 603
Email:	graham.evans@arysta.com

2) **Fine Agrochemicals Limited**

Address:	Hill End House Whittington, Worcester WR5 2RQ UK

Contact: Mr. René Bibars-Reiter
Email: renebr@fine.eu
Telephone number: +44 190 536 1800

1.2.2 Producer or producers of the active substance

For further information please refer to Volume 4 CA-CP (confidential information).

Location of the manufacturing site

For further information please refer to Volume 4 CA-CP (confidential information).

1.2.3 Information relating to the collective provision of dossiers

The EU Daminozide Task Force (an equal partnership between: **Arysta LifeScience Great Britain Limited** (formerly: MacDermid Agricultural Solutions Incorporated, Chemtura Europe Limited and Uniroyal Chemical Limited) and **Fine Agrochemicals Limited**) submitted one dossier for active substance and two dossiers for the representative products - Alar and Dazide Enhance.

1.3 Identity of the active substance

1.3.1 Common name and synonyms

ISO common name: Daminozide (ISO); no synonyms

1.3.2 Chemical name (IUPAC and CA nomenclature)

IUPAC: *N*-dimethylaminosuccinamic acid or 4-(2,2-dimethylhydrazino)-4-oxobutanoic acid

CA: butanedioic acid mono(2,2-dimethylhydrazide)

1.3.3 Producer's development code number

None

1.3.4 CAS, EEC and CIPAC numbers

CAS number: 1596-84-5

CIPAC number: 330

EC number: 216-485-9

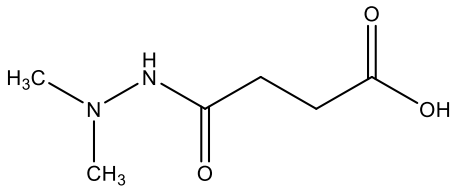
EU Index number: Not allocated

1.3.5 Molecular and structural formulae, molecular mass

Molecular Formula: $C_6H_{12}N_2O_3$

Molecular Mass: 160.2 g/mole

Structural formula



1.3.6 Method of manufacture (synthesis pathway) of the active substance

For further information please refer to Volume 4 CA-CP (confidential information).

1.3.7 Specification of purity of the active substance in g/kg

Minimum purity of daminozide is 990 g/kg.

For further information about specification please refer to Volume 4 CA-CP (confidential information).

1.3.8 Identity and content of additives (such as stabilisers) and impurities

1.3.8.1 Additives

For further information please refer to Volume 4 CA-CP (confidential information).

1.3.8.2 Significant impurities

For further information please refer to Volume 4 CA-CP (confidential information).

1.3.8.3 Relevant impurities

N-nitrosodimethylamine (NDMA)	max 2.0 mg/kg
1,1-Dimethylhydrazide (UDMH)	max 30 mg/kg

1.3.9 Analytical profile of batches

For further information please refer to Volume 4 CA-CP (confidential information).

1.4 Information on the plant protection products

1.4.1 Applicants

1) <u>Arysta LifeScience Great Britain Limited</u>

Registered company address: 3-5 Melville Street
Edinburgh
EH3 7 PE
United Kingdom

Correspondence address: Brooklands Farm
Cheltenham Road
Evesham
Worcestershire
WR11 2LS
United Kingdom

Contact: Mr Graham Evans
Telephone number: +44 1753 555 603
Email: graham.evans@arysta.com

2) Fine Agrochemicals Limited

Address: Hill End House
Whittington
Worcester
WR5 2RQ, UK
Contact: Mr. René Bibars-Reiter
Email: renebr@fine.eu
Telephone number: +44 190 536 1800

1.4.2 Producers of the plant protection products

For further information please refer to Volume 4 CA-CP (confidential information).

Location of the manufacturing site

For further information please refer to Volume 4 CA-CP (confidential information).

1.4.3 Trade name or proposed trade name and producer's development code number of the plant protection Products

1) Trade name: **Alar**
Formulation code: UBI 6899-00

For alternative or earlier names and codes used in the dossier see Volume 4 CA-CP (confidential information).

2) Trade names: **Dazide Enhance** (Fytozide, Imex)
Formulation code: FAL 2400

For alternative or earlier names and codes used in the dossier see Volume 4 CA-CP (confidential information).

1.4.4 Detailed quantitative and qualitative information on the composition of the plant protection products

1.4.4.1 Composition of the plant protection products

Detailed information on the composition of the preparations is confidential and provided in Volume 4 CA-CP (confidential information).

Alar

content of pure active substance:	**850 g/kg**	**(85% w/w)**
limits: ± 25 g/kg	825 - 875 g/kg	82.5 - 87.5 % w/w
content of technical active substance:	858.6 g/kg	(85.86% w/w)
at a minimum purity of the technical active substance of 99.0%		

Dazide Enhance

content of pure active substance:	**851.4 g/kg**	**(85.14% w/w)**
limits: ± 25 g/kg	826.4 - 876.4 g/kg	82.64 - 87.64 % w/w
content of technical active substance:	860.0 g/kg	(86% w/w)
at a minimum purity of the technical active substance of 99.0%		

1.4.4.2 Information on the active substances

Type	**Name/Code Number**
ISO common name	Daminozide
CAS No	1596-84-5
EC No	216-485-9
CIPAC No	330
EU Index No	Not allocated
Salt, ester anion or cation present	Not applicable

1.4.4.3 Information on safeners, synergists and co-formulants

Available information on the formulations components is confidential. Please refer to Volume 4 CA-CP (confidential information).

1.4.5 Type and code of the plant protection products

Water soluble Granule [SG]

1.4.6 Function

Plant Growth Regulator

1.4.7 Field of use envisaged

Horticulture in field and glasshouse situations.

1.4.8 Effects on harmful organisms

Not applicable. The product is a plant growth regulator.

1.5 Detailed uses of the plant protection product

Alar/Dazide Enhance contains daminozide, an acyclobutanedione, which acts as a plant growth regulator reducing internode length and promoting flower production by the inhibition of giberellins and ethylene. Daminozide was one of the first chemicals used as a plant growth regulator with a mode of action which inhibits plant growth. It is taken up by plant foilage, it is systemic, and is currently applied by a foliar spray e.g. via an automated sprayer system such as the Dosatron or via a knapsack sprayer.

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1.5.1 Details of representative uses

PPP (product name/code)	**ALAR/DAZIDE ENHANCE**	**Formulation type:**	**SG**
active substance 1	**Daminozide**	**Conc. of as 1:**	**850 g/kg**
active substance 2	**Not applicable**	**Conc. of as 2:**	**Not applicable**
active substance	**Not applicable**	**Conc. of as:**	**-**
safener	**None**	**Conc. of safener:**	**Not applicable**
synergist	**None**	**Conc. of synergist:**	**Not applicable**
Applicant:	**Arysta LifeScience Great Britain Ltd. /Fine Agrochemicals Limited**	**professional use**	☒
		non professional use	☐
Zone(s):	**EU**		
Verified by MS:	**Y**		

1	2	3	4	5	6	7	8	10	11	12	13	14
Use-No.	**Member state(s)**	**Crop and/ or situation** **(crop destination / purpose of crop)**	**F G or I**	**Pests or Group of pests controlled** (additionally: developmental stages of the pest or pest group)	**Application**			**Application rate**			**PHI** (days)	**Remarks:** e.g. safener/synergist per ha e.g. recommended or mandatory tank mixtures
					Method / Kind	Timing / Growth stage of crop & season	Max. number (min. interval between applications) a) per use b) per crop/ season	kg product / ha a) max. rate per appl. b) max. total rate per crop/season	kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min / max		
1	EU	Ornamentals	G	Plant growth regulator	gantry automated / hand-held	Actively growing plants	a) 5 (7) b) 5 (7)	a) 9.0 b) 45.0	a) 7.65 b) 38.25	500 – 1500	-	
2	EU	Ornamentals	F	Plant growth regulator	hand-held	Actively growing plants	a) 5 (7) b) 5 (7)	a) 5.0 b) 25.0	a) 4.25 b) 21.25	500 – 1500	-	

1.5.2 Details of other uses applied for to support the setting of MRLs for uses beyond the representative uses

No other uses applied for to support the setting of MRLs.

1.5.3 Overview on authorisations in EU Member States

Arysta LifeScience Great Britain Ltd. (wholly owned subsidiary of Arysta LifeScience Inc., formerly known as MacDermid Agricultural Solutions Inc., and before that as Chemtura Corporation**)**

Country	Since	Reg. No.	Product	Crop(s)	F/G	Maximum individual dose kg a.s./ha	Maximum number of treatments
Austria	24-Sept-13	3359	Alar 85 SG	Ornamental plants	G	4.25 kg a.s/ha	12
Belgium	17-Feb-06	9457 P/B	Alar 85 SG	Ornamental plants	G	0.510 kg/hl	5
Belgium	11-Apr-12	10097 P/B	B-Nine	Ornamental plants	G	0.510 kg/hl	5
Cyprus	24-Jul-08	2725	B-Nine SG	Ornamental plants	G	0.425 kg as/ha	3
Denmark	08-Jun-12	558-7	Alar 85 SG	Ornamental plants	G	0.425 kg as/ha	Maximum total dose 12.75 kg as/ha
France	30-Jul-10	2100066	Alar 85 SG / B-Nine SG	Ornamental plants	G	0.425 kg as/hl	3
Greece	19-Jan-11	8194	Alar 85 SG	Ornamental plants	G	0.425 kg as/ha	3
Hungary	26-Dec-08	46094/1981	Alar 85	Alfafa Red Clover	F	4.25 kg as/ha	1
Ireland	Not available	PCS No. 3412	B-NINE SG	Ornamental plants	G	4.25 kg a.s/ha	Maximum total dose 12.75 kg as/ha
Italy	30-Mar-94	8479	Alar 85 SG	Ornamental plants	G	4.25 kg a.s/ha	Maximum total dose 12.75 kg as/ha
Italy	28-Apr-05	12450	B Nine	Ornamental plants	G	4.25 kg a.s/ha	Maximum total dose 12.75 kg as/ha
Italy	27-Mar-09	12770	Alar 85 Gold	Ornamental plants	G	4.25 kg a.s/ha	Maximum total dose 12.75 kg as/ha
Netherlands	26-Jul-95	8589 N	Alar 64 SP	Ornamental plants	G	4.25 kg a.s/ha	Maximum total dose 12.75 kg as/ha
Netherlands	26-Nov-04	12610 N	Alar 85 SG	Ornamental plants	G	0.425 kg as/hl	Maximum total dose 12.75 kg as/ha
Poland	05-Mar-12	R-41/2012	B-Nine 85 SG	Chrysanthemums Poinsettias	G	0.425 kg as/hl	2

ANNEX 1 – BACKGROUND DOCUMENT TO RAC OPINION ON DAMINOZIDE (ISO); 4-(2,2-DIMETHYLHYDRAZINO)-4-OXOBUTANOIC ACID; N-DIMETHYLAMINOSUCCINAMIC ACID

Country	**Since**	**Reg. No.**	**Product**	**Crop(s)**	**F/G**	**Maximum individual dose** kg a.s./ha	**Maximum number of treatments**
Spain	30-Sept-15	15465	B Nine	Ornamental plants	G	0.6375 kg as/hl	-
Sweden	28-Feb-16	4329	Alar 85 SG	Ornamental plants	G	4.25 kg a.s/ha	-
United Kingdom	07-Apr-09 11-May-09	M14434 M 14435	B NINE SG	Ornamental plants	G	4.25 kg a.s/ha	Maximum total dose 12.75 kg as/ha

Fine Agrochemicals Limited

Country	Since	Reg. No.	Product	Crop(s)	F/G	Maximum individual dose kg a.s./ha	Maximum number of treatments
Austria	29/06/2012	3208	Dazide Enhance	Pot Chrysanthemums	G	4.25	2
				Cut chrysanthemums	G	5.1	3
				Kalanchoe	G	3.825	3
				Ornamentals	G	7.65	5
Belgium	07/02/2006	9455P/B	Dazide Enhance	Azalea	G	7.65	5
				Pot Chrysanthemums	G	3.188	5
				Spray Chrysanthemums	G	1.912	5
				Hortensia	G	7.65	5
				Lobelia	G	3.825	5
				Verbena	G	3.825	5
				Ornamentals	G	7.65	5
Denmark	01/01/2004	544-6	Dazide Enhance	Pot plants	G	7.65	5
				Bedding plants	G	7.65	5
				Cut chrysanthemums and Sunflowers	G	6.375	2
France	30/07/2010	2100067	Dazide Enhance	Pot chrysanthemums	G	4.25	2
				Cut chrysanthemums	G	4.25	3
				Cut Chrysanthemums (spray)	G	1.06	2
				Sunflower	G	3.4	3
				Ornamentals/ bedding plants	G	4.25	3
				Kalanchoe	G	2.55	3
				Hortensia	G	3.4	3
				Petunia and Calibrachoa	G	4.25	3
				Flower and foliage crops	G	4.25	3
Germany	28/02/2011	ZA1 006273-00/00	Dazide Enhance	Pot Chrysanthemums	G	4.25	2
				Cut chrysanthemums	G	5.1	3
				Kalanchoe	G	3.825	3
				Ornamentals	G	7.65	5
Greece	30/01/2011	8195	Dazide	Pot chrysanthemums	G	8.5	2

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Country	**Since**	**Reg. No.**	**Product**	**Crop(s)**	**F/G**	**Maximum individual dose** kg a.s./ha	**Maximum number of treatments**
			Enhance	Cut chrysanthemum	G	5.1 6.375	3 2
				Chrysanthemum Shoesmith and Rivalry sports	G	1.1475	3
				Sunflower	G	5.1	3
				Ornamental potted plants (including Aster, Brassica, Cosmos, Dicenta, Lobelia, Nemesia, Phlox, Salvia, Tagetes, Viola and Zinnia).	G	7.65	5
				Kalanchoe	G	3.825	3
				Hydrangea	G	5.1	3
				Petunia	G	7.65	5
				Potted azalea	G	2.125	1
Ireland	07/08/2009	03852	Dazide Enhance	Pot Chrysanthemums	G	6.375	2
				Standard Chrysanthemums	G	6.375	3
				Spray Chrysanthemums	G	1.594	2
				Sunflowers	G	5.1	3
				Ornamentals and Bedding Plants	G	6.375	5
				Kalanchoe	G	3.825	3
				Hortensia	G	5.1	3
				Petunia and Calibrachoa	G	7.65	5
Italy	30/05/2007	12455	Dazide Enhance	Pot chrysanthemum	G	6.375	2
				Chrysanthemum	G	5.1	3
				Spray chrysanthemum	G	1.594	2
				Sunflower	G	5.1	3
				Pot ornamentals and bedding plants	G	6.375	5
				Kalanchoe	G	3.825	3
				Hortensia	G	5.1	
				Petunia and Calibrachoa	G	7.65	5

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Country	**Since**	**Reg. No.**	**Product**	**Crop(s)**	**F/G**	**Maximum individual dose** kg a.s./ha	**Maximum number of treatments**
The Netherlands	31/03/2004	8962	Dazide Enhance	Potted and bedding plants (such as Aster, Azalea, Brassica, Cosmos, Dicentra, Lobelia, Nemesia, Phlox, Salvia, Tagetes, Viola, Zinnia)	G	5.1	5
				Kalanchoe	G	2.55	3
				Hortensia	G	3.4	3
				Petunia/ Calibrachoa	G	5.1	5
				Sunflower (pot and cut plants)	G	3.4	3
				Pot chrysanthemum	G	4.25	2
				Cut chrysanthemum	G	4.25	3
				Cut chrysanthemums (Euro – fast growing)	G	6.375	2
				Chrysanthemums (Shoesmith, Rivalry)	G	1.7	3
Poland	18/04/2011	8216	Dazide Enhance	Cut chrysanthemums	G	2.55	2
				Pot chrysanthemum	G	4.25 2.125	2 4
Portugal	15/02/2006	3746	Dazide Enhance	Cut chrysanthemum	G	6.375	2
				Chrysanthemum (large flower)	G	6.375	3
				Chrysanthemum (spray)	G	1.594	2
				Sunflower	G	5.1	3
				Ornamentals and bedding plants	G	6.375	5
				Kalanchoe	G	3.825	3
				Hortensia	G	5.1	3
				Petunia and Calibrachoa	G	7.65	5
Spain	03/11/2011	24.977	Dazide Enhance	Azalea	G	3.315	5
				Chrysanthemum	G	2.933	5
				Gardenia	G	3.315	5

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Country	**Since**	**Reg. No.**	**Product**	**Crop(s)**	**F/G**	**Maximum individual dose** kg a.s./ha	**Maximum number of treatments**
				Hortensia	G	3.315	5
				Herbaceous ornamentals	G	9.563	5
				Poinsettia	G	3.315	5
				Nurseries	G	3.315	5
Sweden	14/12/2011	5033	Dazide Enhance	Pot chrysanthemums	G	4.25	2
				Cut chrysanthemums	G	4.25	3
				Cut chrysanthemums spray	G	1.063	2
				Ornamentals and bedding plants	G	7.65	3
				Kalanchoe	G	3.825	3
				Ornamentals	G	7.65	5
UK	18/02/2004	MAPP 14433	Dazide Enhance	Pot Chrysanthemums	G	6.375	2
				Standard Chrysanthemums	G	6.375	3
				Spray Chrysanthemums	G	1.594	2
				Sunflowers	G	5.1	3
				Ornamentals and Bedding Plants	G	6.375	5
				Kalanchoe	G	3.825	3
				Hortensia	G	5.1	3
				Petunia and Calibrachoa	G	7.65	5
				Ornamentals	G	7.65	5

LEVEL 2

ANNEX 1 – BACKGROUND DOCUMENT TO RAC OPINION ON DAMINOZIDE (ISO); 4-(2,2-DIMETHYLHYDRAZINO)-4-OXOBUTANOIC ACID; N-DIMETHYLAMINOSUCCINAMIC ACID

2 SUMMARY OF ACTIVE SUBSTANCE HAZARD AND OF PRODUCT RISK ASSESSMENT

2.1 Identity

2.1.1 Summary of identity

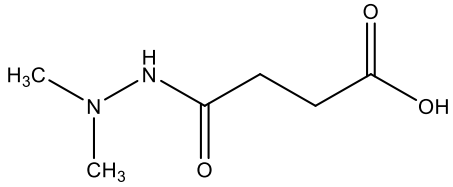
Daminozide (ISO) is a plant growth regulator and belongs to the family of growth retardants.

Chemical name (IUPAC): *N*-dimethylaminosuccinamic acid or 4-(2,2-dimethylhydrazino)-4-oxobutanoic acid

Molecular formula: C6H12N2O3

Molecular mass: 160.1711 g/mole

Structural formula:



Relevant impurities: N-nitrosodimethylamine (NDMA) max 2.0 mg/kg

1,1-Dimethylhydrazide (UDMH) max 30 mg/kg

Batches: full scale production (confidential; see Vol 4 of RAR)

Minimum purity: 990 g/kg

Additives: none

Isomers: daminozide is not mixture of isomers

2.2 Physical and chemical properties [Equivalent to Section 7 of the CLH report template]

2.2.1 Summary of physical and chemical properties of the active substance

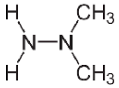
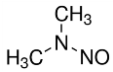
Table 1: Summary of physical and chemical properties of the active substance

Property	Value (purity)	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	Solid at 20.5°C with the sub-classification crystalline consisting of small fine approximately cubic shaped crystals. Slightly off-white with Munsell Notation N 9.5/. (998 g/kg and 999 g/kg)	Riggs, A.S. (2008)	Visual
Melting/~~freezing~~ point	153-154.5ºC (999 g/kg)	Riggs, A.S. (2010)	Measured
Boiling point		Riggs, A.S. (2003)	The boiling point could not be determined as the test material decomposed in the range of 142-145°C.
Relative density			Not a requirement according to 283/2014
Vapour pressure	1.5 x 10^{-6} Pa at 25°C (997 g/kg) very slightly volatile	Tremain, S.P. (2001)	Measured
Surface tension	69.8 mN/m at 25°C (999 g/kg) 0.1% solution of Daminozide in Milli-RO water	Thompson, A.K. (1999)	Measured
Water solubility	128 g/L at 20°C and pH 4 (1000 g/kg) readily soluble	Friedlander, B.T. (2011)	Measured
Partition coefficient n-octanol/water	Log P_{ow}: -1.53 at 20°C and pH 3 (1000 g/kg) No possibility for bioaccumulation	Riggs, A.S. (2011)	Measured
Henry's law constant	1.0 x 10^{-9} Pa x m^3 x mol^{-1} at 25°C (calculated) very slightly volatile	Liney, P.; Miles, D. (2014)	Calculated using water solubility and vapour pressure results
Flash point			Not relevant, as test substance is a solid with a melting point > 40°C
Flammability	Not highly flammable (999 g/kg)	Jackson, W.A. (1999)	Measured

Property	Value (purity)	Reference	Comment (e.g. measured or estimated)
		Tremain, S.P. (1999)	
Explosive properties	Mechanical sensitivity (friction): Negative Mechanical sensitivity (shock): Negative Thermal sensitivity: Negative The test material does not possess explosive properties. (999 g/kg)	Jackson, W.A. (1999) Tremain, S.P. (1999)	Measured
Self-ignition temperature	no self-ignition below melting point about 154°C (999 g/kg)	Jackson, W.A. (1999)	Measured
Oxidising properties	None of the test substance/cellulose mixtures burned to completion. The test material does not possess oxidising properties. (997 g/kg)	Tremain, S.P. (1999) Flack, I. (2001) Cowlyn, N. (2014)	Measured
Granulometry			Not a requirement according to 283/2014
Solubility in organic solvents and identity of relevant degradation products	Solubility at 20°C: readily soluble in acetone and methanol Acetone: 1.61 g/L Methanol: 48.0 g/L Solubility at 25°C: slightly soluble in toluene and moderately soluble in dichloromethane Toluene: < 0.01 g/L Solubility at 20°C: moderately soluble in ethyl acetate Ethyl acetate: 0.27 g/L (999 g/kg - 1001 g/kg)	Friedlander, B.T. (2011b) Parsons, A.H. (2006) Thompson, A.K. (1999a)	Measured
Dissociation constant	pKa = 4.68 at 20°C (993 g/kg)	Tang, C.L.; Rose, K.G. (1988)	Measured
Viscosity			Not a requirement according to 283/2014

Spectra (UV/VIS, IR, NMR, MS)

UV, IR, NMR and Mass spectrum of active substance	UV-Vis OPPTS 830.7050	2007-11-05 994 g/kg	pH Maxima (nm) Molar Absorption Coefficient (ε) 1.95 198 951 L mol^{-1} cm^{-1} 6.99 191 6520 L mol^{-1} cm^{-1} 10.10 192 6966 L mol^{-1} cm^{-1} No absorption at wavelengths above 290 nm.	No extinction coefficients presented in the DAR. Acceptable.	Y	Kelly, K. (2011) J18897
	IR	S-3410 995 g/kg	The spectrum was consistent with the structure of daminozide and contained the following signals: OH & NH stretch, C=O stretch, N-H bend, CH bend, OH bend, C-O stretch, C-N stretch, C-C stretch, CH rock, NH rock, alcohol O-H, amine N-H, alkane C-H, aldehyde C-H, aldehyde C=O, ketone C=O, ester C-O, ester C=O, amide C=O, amide C-O	No peak wave numbers or assignments presented in the DAR. Acceptable.	Y	Knowles, R.J. (2006) J15709
	NMR	081028092 > 990 g/kg	The ^{1}H and ^{13}C NMR spectra for the test substance were consistent with the structure of daminozide and practically identical to the spectra obtained for a daminozide reference standard. The chemical shift values are presented below and detailed structural assignments are presented in the study report. ^{1}H NMR Signal NMR Shift (ppm) 2.50 > 2.57 2.15 > 2.41 2.43 > 2.44 8.26 ^{13}C NMR Signal NMR Shift (ppm) 39.523 > 41.193 27.415 > 29.855 47.218 > 48.432 169.192 > 174.892	The previous study was not performed to GLP. Acceptable.	Y	Riggs, A.S. (2010b) GRL-12900
	MS	Daminozide	The following fragmentation pathway has been assigned for the mass spectrum of daminozide. Fragment (m/z) Data 160 Molecular ion	No fragmentation data were presented in the previous study.	N	Parsons, A.H.; White, G.A. (Unknown) 196715425

			143 Loss of -OH group 118 Loss of N $(CH_2)_2$ group 101 Loss of OH-C=O plus CH_3 100 Loss of NH-C-$(CH_3)_2$ 73 Cleavage of O=C(OH)-CH_2-CH_2 59 Base peak from NH-N-$(CH_3)_2$ 45 O=C-OH 44 N-$(CH_3)_2$	GLP status, batch, purity and date of study are unknown. However, identification of daminozide by MS seems to be acceptable.		
Spectra of relevant impurities UDMH 	UV-Vis OECD 101	UDMH BCBJ7409V 998 g/kg	The UV/Vis spectra only show minor absorbance at low wavelengths for the neutral and basic solutions; however the spectra obtained were consistent with the assigned structure of UDMH.	No spectral data for UDMH previously presented. Acceptable.	Y	Cowlyn, N. (2014a) FDD0119
	IR	UDMH BCBJ7409V 998 g/kg	Frequency (cm^{-1}) — Assignment 3100 - 3300 N-H stretches 2700 - 3000 C-H (alkyl) stretches 1601 NH_2 deformation 1400 - 1500 CH_3 deformation 1319 C-N stretch < 1250 NH_2 deformation, CH_3 vibrations The IR spectrum was consistent with the assigned structure of UDMH.	No spectral data for UDMH previously presented. Acceptable.	Y	Cowlyn, N. (2014a) FDD0119
	NMR	UDMH BCBJ7409V 998 g/kg	Chemical Shift — Number of protons — Assignment (ppm) 2.4 — 6 — C**H**$_3$ group 3.0 — 2 — N**H**$_2$ group 7.3 — - — Solvent The proton NMR spectrum was consistent with the assigned structure of UDMH.	No spectral data for UDMH previously presented. Acceptable.	Y	Cowlyn, N. (2014a) FDD0119
	MS	UDMH BCBJ7409V 998 g/kg	Molecular Ion - m/z 61. No assignable fragments or adducts were observed. The mass spectrum was consistent with the assigned structure of UDMH.	No spectral data for UDMH previously presented. Acceptable.	Y	Cowlyn, N. (2014a) FDD0119
NDMA 	UV-Vis OECD 101	NDMA 30924 972 g/kg	pH — λ_{max} (nm) — (ε) 7.2 — 227 — 7600 — 333 — 92.7 1.3 — 227 — 7630 — 332 — 117 12.8 — 228 — 7570	No spectral data for NDMA previously presented. Acceptable.	Y	Cowlyn, N. (2014b) FDD0118

			332 99.8 The UV/Vis spectra were consistent with the assigned structure of NDMA.			
	IR	NDMA 30924 972 g/kg	Frequency (cm^{-1}) Assignment 2840 - 3000 C-H (alkyl stretches 1000 - 1400 CH_3 deformation C-N stretch N=O stretch < 1000 Skeletal vibrations The IR spectrum was consistent with the assigned structure of NDMA.	No spectral data for NDMA previously presented. Acceptable.	Y	Cowlyn, N. (2014b) FDD0118
	NMR	NDMA 30924 972 g/kg	Chemical Shift Assignment (ppm) 3.06 C**H**$_3$ group 3.78 N**H**$_2$ group 7.27 Solvent The proton NMR spectrum was consistent with the assigned structure of NDMA.	No spectral data for NDMA previously presented. Acceptable.	Y	Cowlyn, N. (2014b) FDD0118
	MS	NDMA 30924 972 g/kg	Molecular Ion - m/z 75. No assignable fragments or adducts were observed. The mass spectrum was consistent with the assigned structure of NDMA.	No spectral data for NDMA previously presented. Acceptable.	Y	Cowlyn, N. (2014b) FDD0118

2.2.1.1 Evaluation of physical hazards [equivalent to section 8 of the CLH report template]

2.2.1.1.1 Explosives [equivalent to section 8.1 of the CLH report template]

Table 2: Summary table of studies on explosive properties

Method	Results	Remarks	Reference
EC A.14	no explosive properties	Purity: 999 g/kg Material: ? Batch: ? GLP: ?	Jackson, W.A. (1999b) HT99/196 (197) (DAR addendum Volume 3, Annex B, June 2002)
EC A.14	Mechanical sensitivity (friction): Negative Mechanical sensitivity (shock): Negative Thermal sensitivity: Negative The test material does not possess explosive properties. (not explosive)	Purity: ? Material: technical Batch: 903M014 SI 6956 GLP: yes	Tremain, S.P. (1999) 666/022

2.2.1.1.1.1 Short summary and overall relevance of the provided information on explosive properties

Two studies were provided on explosive properties of technical daminozide. Second was acceptable and negative.

Daminozide is not considered explosive. Results are acceptable according to CLP criteria.

2.2.1.1.1.2 Comparison with the CLP criteria

Thermal sensitivity and mechanical sensitivity (shock + friction) were negative in test. According to the CLP criteria Daminozide is not explosive.

2.2.1.1.1.3 Conclusion on classification and labelling for explosive properties

Daminozide is not classifiable as explosive.

2.2.1.1.2 Flammable gases (including chemically unstable gases) [equivalent to section 8.2 of the CLH report template]

Not tested/Not relevant.

2.2.1.1.3 Oxidizing gases [equivalent to section 8.3 of the CLH report template]

Not tested/Not relevant.

2.2.1.1.4 Gases under pressure [equivalent to section 8.4 of the CLH report template]

Not tested/Not relevant.

2.2.1.1.5 Flammable liquids [equivalent to section 8.5 of the CLH report template]

Not tested/Not relevant.

2.2.1.1.6 Flammable solids [equivalent to section 8.6 of the CLH report template]

Table 3: Summary table of studies on flammable solids

Method	Results	Remarks	Reference
EC A.10	not highly flammable in the sense of EC A.10	Purity: 999 g/kg Material: ? Batch: ? GLP: ?	Jackson, W.A. (1999b) HT99/196 (197) (DAR addendum Volume 3, Annex B, June 2002)
EC A.10	not highly flammable in the sense of EC A.10 (not flammable) The flammability was determined by measuring the burning rate of test material prepared as a pile of set dimensions. **Preliminary screening test:** The pile ignited and burnt with a blue/orange flame, which self-extinguished 12 seconds after Bunsen flame was removed, without propagating combustion. The result of the preliminary screening test obviated the need to perform the main test. **Moisture content:** Mean 0.509 % w/w	Purity: ? Material: technical Batch: 903M014 SI 6956 GLP: yes	Tremain, S.P. (1999) 666/022

2.2.1.1.6.1 Short summary and overall relevance of the provided information on flammable solids

Two studies were provided on flammability of technical daminozide. Second was acceptable and negative. Preliminary screening test was negative. Daminozide is not considered highly flammable. Results are acceptable according to CLP criteria.

2.2.1.1.6.1 Comparison with the CLP criteria

According to the CLP criteria Daminozide is not flammable.

2.2.1.1.6.2 Conclusion on classification and labelling for flammable solids

Daminozide should not be labelled flammable.

2.2.1.1.7 Self-reactive substances [equivalent to section 8.7 of the CLH report template]

Not tested/Not relevant. There are no chemical groups present in the molecule associated with explosive or self reactive properties.

2.2.1.1.8 Pyrophoric liquids [equivalent to section 8.8 of the CLH report template]

Not tested/Not relevant.

2.2.1.1.9 Pyrophoric solids [equivalent to section 8.9 of the CLH report template]

Not tested/Not relevant. The substance does not ignitate spontaneously on coming into contact with air at normal

temperatures (the substance is known to be stable at room temperature for prolonged periods of time (at least one day)).

2.2.1.1.10 Self-heating substances [equivalent to section 8.10 of the CLH report template]

Table 4: Summary table of studies on self-heating substances

Method	Results	Remarks	Reference
EC A.16	no self-ignition below melting point (about 154°C) (not auto-flammable)	Purity: 999 g/kg Material: ? Batch: ? GLP: ?	Jackson, W.A. (1999b) HT99/196 (197) (DAR addendum Volume 3, Annex B, June 2002)

2.2.1.1.10.1 Short summary and overall relevance of the provided information on self-heating substances

One study on auto-ignition was provided. The substance has a low melting temperature (<154°C) and should not be considered for classification in this hazard class according to the CLP Guidance. New study is not available, the results are still acceptable. Results are acceptable according to CLP criteria.

2.2.1.1.10.2 Comparison with the CLP criteria

The substance has a low melting temperature (<154°C) and should not be considered for classification in this hazard class according to the CLP Guidance. According to the CLP criteria Daminozide is not auto-flammable.

2.2.1.1.10.3 Conclusion on classification and labelling for self-heating substances

Not relevant.

2.2.1.1.11 Substances which in contact with water emit flammable gases *[equivalent to section 8.11 of the CLH report template]*

Not tested/Not relevant. The substance is known to be soluble in water to form a stable mixture.

2.2.1.1.12 Oxidizing liquids [equivalent to section 8.12 of the CLH report template]

Not tested/Not relevant.

2.2.1.1.13 Oxidizing solids [equivalent to section 8.13 of the CLH report template]

Table 5: Summary table of studies on oxidising solids

Method	Results	Remarks	Reference
EC A.17	None of the test substance/cellulose mixtures burned to completion. The test material does not possess oxidising properties. (not oxidising) **Maximum burning rate of test material mixtures:** 0.877 mm/s	Purity: ? Material: technical Batch: 903M014 SI 6956 GLP: yes	Tremain, S.P. (1999) 666/022

Method	Results	Remarks	Reference
	Maximum burning rate of reference mixtures: 1.408 mm/s		
EC A.17	no oxidising properties	Purity: 999 g/kg Material: technical Batch: ZJ 00-05-14 GLP: yes (Purity: 997 g/kg Material: technical Batch: 009M009 GLP: yes)	Comb, A.L. (2001a) FNA102/014401 (Flack, I. (2001a) URO 016/012463) (DAR addendum Volume 3, Annex B, June 2002)
EC A.17	None of the test substance/cellulose mixtures burned to completion. No further testing was therefore necessary. The test material does not possess oxidising properties. (not oxidising) **Test substance/cellulose ratio:** 2:1; 1:1 and 1:2 **Duration of combustion:** 112; 78 and 75 seconds **Observations:** Did not burn to completion - burned with a gentle yellow flame, approximately 2 mm remained unburnt at the base of the cone	Purity: 997 g/kg Material: technical Batch: 4A27-21DA GLP: yes	Cowlyn, N. (2014c) FDD0116

2.2.1.1.13.1 Short summary and overall relevance of the provided information on oxidising solids

Three studies were provided on oxidizing properties of technical daminozide. All were acceptable and negative.

First study - daminozide technical has been determined not to have oxidising properties as the test material/cellulose mixtures failed to propagate combustion at a rate greater than or equal to that of the barium nitrate/cellulose mixtures.

Second study was evaluated in Monograph and in DAR addendum (2002).

Third study - none of the test substance/cellulose mixtures burned to completion. No further testing was therefore necessary.The test material does not possess oxidising properties. Results are acceptable according to CLP criteria.

2.2.1.1.13.2 Comparison with the CLP criteria

The substance should not be considered for classification in this hazard class according to the CLP Guidance.

2.2.1.1.13.3 Conclusion on classification and labelling for oxidising solids

Daminozide is not classifiable as oxidizing solids.

2.2.1.1.14 Organic peroxides [equivalent to section 8.14 of the CLH report template]

Not tested/Not relevant.

2.2.1.1.15 Corrosive to metals [equivalent to section 8.15 of the CLH report template]

Not tested/Not relevant.

2.2.2 Summary of physical and chemical properties of the plant protection products

Alar

Alar is a water soluble granule (SG) formulation containing 85% w/w daminozide as active substance. It is a white, granular solid and the pH of a 1% dilution is 4.04. The product is neither flammable nor auto-flammable and does not possess oxidizing or explosive properties. Alar has good dilution, wettability, flowability and attrition characteristics, is 'nearly dust free' and does not produce excessive amounts of foam. The product has been demonstrated to be stable in studies at 54°C for 14 days and room temperature for 2 years, with no significant loss of active substance content. The packaging of the product remained free from any corrosion or degradation for the duration of the stability studies and the shelf life of the product is 24 months. The technical properties of ALAR indicate that no particular problems are expected when it is used as recommended and there are no implications for classification.

Dazide Enhance

Dazide Enhance is a water soluble granule (SG) formulation containing 85.1% w/w daminozide as active substance. It is a white, fine granular solid and the pH of a 1% dilution is 4.1. The product is neither flammable nor auto-flammable and does not possess oxidizing or explosive properties. Dazide Enhance has good dilution, wettability, flowability and attrition characteristics, is 'nearly dust free' and does not produce excessive amounts of foam. The product has been demonstrated to be stable in studies at 54°C for 14 days and room temperature for 2 years, with no significant loss of active substance content. The packaging of the product remained free from any corrosion or degradation for the duration of the stability studies and the shelf life of the product is 24 months. The technical properties of Dazide Enhance indicate that no particular problems are expected when it is used as recommended and there are no implications for classification.

RAC evaluation of physical hazards

Summary of the Dossier Submitter's proposal

The dossier submitter (DS) proposed no classification of daminozide for physical hazards on the basis of the following data:

Property and Method	Results	Remarks	Reference
Explosives			
EC A.14	Not explosive	Purity: 99.9%	Jackson, 1999b
EC A.14	Not explosive	Purity: unknown	Tremain, 1999
Flammable solids			

EC A.10	Not highly flammable	Purity: 99.9%	Jackson, 1999b
EC A.10	Not highly flammable	Purity: unknown	Tremain, 1999
Self-reactive substances	Not self-reactive	Screening method: there are no chemical groups present in the molecule associated with explosive or self-reactive properties.	
Pyrophoric solids	Not pyrophoric properties	Statement: Based on experience in manufacturing and handling	
Self-heating A.16	No self-ignition below melting point (about 154°C) (not auto-flammable)	Purity: 99.9%	Jackson, 1999b
Substance which in contact with water emits flammable gas		The substance is known to be soluble in water to form a stable mixture	
Oxidising solids			
A.17	Not oxidising	Purity: unknown Did not burn to completion	Tremain, 1999
A.17	Not oxidising	Purity: 99.9%	Comb, 2001a
A.17	Not oxidising	Purity: 99.7% Did not burn to completion	Cowlyn, 2014c
Substance corrosive to metals		Not relevant	

Comments received during public consultation

There were no comments provided.

Assessment and comparison with the classification criteria

Explosive

Based on the results of the screening procedure daminozide was identified as potentially explosives and the acceptance procedure should have been performed. Daminozide's chemical structure contains a feature (N-N) associated with explosive properties, its oxygen balance is -149.8, i.e. above the cut off value of -200, no information on the decomposition energy or onset temperature is included in the CLH report.

Daminozide was tested negative in two A.14 studies, however the substance was not tested according to the test series 2 to 8 of the UN RTGD (acceptance procedure), hence no conclusion can be drawn based on these studies alone.

Based on the available information, **RAC is unable to conclude on this hazard class due to lack of data.**

Flammable solids

Daminozide was considered as 'not highly flammable' in two A.10, therefore the **classification as flammable solids is not warranted**, see R.7.1.10.3 of Guidance on Information Requirements and Chemical Safety Assessment (R.7a).

Self-reactive substances

RAC disagrees with the DS on the assessment of the presence of groups associated with explosive or self-reactive properties. As no additional information were included in the CLH dossier, **RAC is unable to conclude on this hazard class due to lack of data**.

Pyrophoric solids

Daminozide is known to be stable in contact with air at room temperature for prolonged periods of time (days) and hence, **it does not warrant classification as a pyrophoric solid**.

Self-heating substances

Daminozide was tested for self-heating properties in an EC A.16 study (Jackson, 1999b), and it did not undergo self-ignition below its melting point of 154°C. In addition, substances with a melting point below 160°C should not be considered for classification in this hazard class, see CLP guidance 2.11.4.2.

In conclusion, **daminozide does not warrant classification as a self-heating substance.**

Substances which in contact with water emit flammable gases

Daminozide does not contain metals or metalloids and it is known to be soluble in water to form a stable mixture. Consequently, **daminozide does not warrant classification as a substance which emits flammable gases in contact with water**.

Oxidising solids

Daminozide does not contain fluorine or chlorine, all oxygen atoms in the structure are chemically bound to only carbon or hydrogen. Therefore, **daminozide does not warrant classification as an oxidising solid.**

Organic peroxides

The substance **does not contain peroxide groups, and therefore classification is not warranted**.

Substance corrosive to metals

The DS considered this hazard class as 'not relevant' without providing a justification. Daminozide is a solid with a melting point above 55ºC, thus it is not within the scope of this hazard class (liquids and solids which may become liquids during transport). Therefore, **daminozide does not meet the criteria for classification as corrosive to metals**.

In conclusion, RAC supports the proposal of the DS for **no classification of daminozide as flammable solids, pyrophoric solids, self-heating substances, substances which in contact with water emit flammable gases, organic peroxides, oxidising solids and substance corrosive to metals**. RAC is unable to conclude on the following hazard classes due to lack of/ insufficient data: **explosives and self-reactive substance**.

2.3 Data on application and efficacy

Daminozide is a plant growth regulator reducing internode length and promoting flower production by the inhibition of gibberellins and ethylene**.**

2.3.1 Summary of effectiveness

Available efficacy data show that daminozide acts as a plant growth regulator to produce more robust plants. Foliage tends to be greener and the plants more able to withstand drought and transport stresses. The period of saleability of many plant types can be extended.

2.3.2 Summary of information on the development of resistance

The proposed use of daminozide is a plant growth regulator. As daminozide is not used for the control of pests, weeds or fungi, the development of resistance is not anticipated from its use.

2.3.3 Summary of adverse effects on treated crops

There are no adverse effects on treated crops.

Response to treatment with daminozide products differs depending on the variety, stage of growth and physiological condition of the plant.

2.3.4 Summary of observations on other undesirable or unintended side-effects

No undesirable or unintended side-effects have been observed.

2.4 Further information

2.4.1 Summary of methods and precautions concerning handling, storage, transport or fire

For information on active substance please see Volume 3 CA_B-4.

For information on representative formulations please see Volumes 3 CP_B-4.

2.4.2 Summary of procedures for destruction or decontamination

For information on active substance please see Volume 3 CA_B-4.

For information on representative formulations please see Volumes 3 CP_B-4.

2.4.3 Summary of emergency measures in case of an accident

For information on active substance please see Volume 3 CA_B-4.

For information on representative formulations please see Volumes 3 CP_B-4.

2.5 Methods of analysis

2.5.1 Methods used for the generation of pre-approval data

Adequate methods are available for the generation of pre-approval data required for the risk assessment analysis.
All data provided are acceptable. No further data are required.

2.5.2 Methods for post control and monitoring purposes

Plants and plant products

Considering that the use of daminozide is restricted to non-consumable crops and that residues are not defined in commodities of plant and animal origin, methods for the determination of daminozide residues in or on food and feed of plant and animal origin are not required.

Food of animal origin

Considering that the use of daminozide is restricted to non-consumable crops and that residues are not defined in commodities of plant and animal origin, methods for the determination of daminozide residues in or on food and feed of plant and animal origin are not required.

Soil

Daminozide was determined in soil by high performance liquid chromatography with tandem mass specific detection (HPLC-MS/MS). Method validation meets EU requirements in all respects and the method is considered suitable for monitoring purposes. The LOQ is 0.05 mg/kg.

Water

Daminozide was determined in surface water by high performance liquid chromatography with tandem mass specific detection (HPLC-MS/MS). Method validation meets EU requirements in all respects and the method is considered suitable for monitoring purposes. The LOQ is 0.1 µg/L. Independent laboratory validations (ILVs) were also successfully conducted for drinking water samples.

Air

Daminozide and UDMH were determined in air by high performance liquid chromatography with tandem mass specific detection (HPLC-MS/MS). The LOQ for daminozide is 160 µg/m^3 and for UDMH is 0.025 µg/m^3.
Daminozide: Validation of the method is not sufficient (LOQ is not low enough).
UDMH: Method validation meets EU requirements in all respects and the method is considered suitable for monitoring purposes.

Body fluids and tissues

According to guideline SANCO/825/00 rev. 8.1 method of analysis is not required if active substance is not classified as either toxic or highly toxic nor is classified according to CLP as follows: Acute toxicity (cat. 1 - 3), CMR (cat. 1) or STOT (cat. 1). On the contrary under regulation 1107/2009 this method is always required. Therefore the analysis in body fluids and tissues is identified a data requirement. Method is ongoing and expected Q4 2018.

2.6 Effects on human and animal health

2.6.1 Summary of absorption, distribution, metabolism and excretion in mammals [equivalent to section

9 of the CLH report template]

Table 6: Summary table of toxicokinetic studies

Method	Results	Remarks	Reference
Toxicokinetics Rat (2/sex) Oral route: by gavage, pre-treatment with 1 mg of non-radiolabelled Alar, subsequently 1.21 mg of ^{14}C-Alar (average dose) No OECD TG	Excretion via faeces (70%), urine (24%), $^{14}CO_2$ (2.4%), complete within 48 h At time of sacrifice: 96% of the radioactivity eliminated, 1.1% retained in organs (liver: 0.15%), 0.83% in carcass Absorption: at least 28% No differences between sexes	Test material: ^{14}C-Alar Radiochemical purity: 98% Deviations from OECD TG 417: study design with high and low dose administration is missing; purity of the unlabelled test compound was not stated **Acceptable study**	*Anonymous (1966)*
Toxicokinetics Miniature swine (3 animals/sex) Oral route: group 1: 5 mg of [^{14}C-methyl]-daminozide/kg bw group 2: pre-treatment with 5 mg of unlabelled daminozide for 10 days, subsequently 5 mg of [^{14}C-methyl]-daminozide/kg bw No OECD TG	Excretion: urine (14%, within 24 h), faeces (59%, within 48h) Absorption: at least 14% The highest levels of radiolabel was found in the liver (0.043 and 0.055 eq/kg for a single dose and pre-treatment, respectively) UDMH and NDMA were identified as major and minor metabolite, respectively	Test material: daminozide Purity: not stated **Supplementary study**	*Anonymous (1987)*
Metabolism study Miniature swine Samples from the previous study *Anonymous, 1987)* No OECD TG	UDMH was detected at a level of almost 0.001 mg eq/kg in a sample of 15 g of liver	Test material: daminozide Purity: not stated **Supplementary study**	*Anonymous (1987)*

Method	Results	Remarks	Reference
Toxicokinetics Rat (5 male F344) Oral: by gavage: a single dose of 1 mg ^{14}C- daminozide/kg bw No OECD TG	Excretion: urine (47%), faeces (32%), $^{14}CO_2$ (7%), exhaled volatile compounds (<1%) Absorption: at least 57% At time of sacrifice: 0.18% retained in liver, 0.10% in blood, 2.2% in carcass	Test material: daminozide Purity: 98.5% unlabelled; 97% labelled Deviations from OECD TG 417: study design with high and low dose administration is missing; total recovery of the radiolabel was below 90% ; kidneys, muscle, and fat were not examined **Acceptable study**	*Anonymous (1993)*
In vitro comparative metabolism study 5 and 50 µM of ^{14}C-daminozide incubated with mouse, rat, dog, and human hepatocytes for 0, 0.5, 1, and 3 h No existing guideline	Using LC-MS/MS, only NDMA was detected in mouse, rat and human treated as well as control (non-hepatocyte) samples. Using radio-HPLC, only UDMH was identified at levels below the limit of quantification (<1%) in all species in the treated as well as control (non-hepatocyte) samples.	Test material: ^{14}C-daminozide Radiochemical purity: >98% **The study of limited validity** (*see summary 2.6.1.1*)	*Anonymous (2017)*

2.6.1.1 Short summary and overall relevance of the provided toxicokinetic information on the proposed classification(s)

Toxicokinetic studies in rats and minipigs (miniature swine) were submitted. However, only single dose studies were performed. A repeated dose study is not available.

Absorption

Minipigs: In male and female miniature swine that were administered 5 mg radio labelled daminozide/kg bw, absorption was established to be at least 14% and was not significantly different in animals that were pre-treated with 10 daily doses of 5 mg unlabelled daminozide/kg bw.

Rats: In male rats that were administered a single dose of 1 mg radiolabelled daminozide/kg bw p.o., absorption was at least 57%. Absorption of at least 28% was established in male and female rats that were administered an oral dose of 1.21 mg radio labelled daminozide/kg bw. Since differences in oral absorption in rats were observed after oral administration (55% and 28%, respectively), information on the oral absorption at a relevant dose level (NOAEL used for risk assessment) is required. A new study (*Anonymous, 1999;* not provided by the applicant) with a single dose administration at 45 mg/kg bw was conducted. From this study, based on urinary and respiratory excretion, an oral absorption value of 35% was adopted. The relative urinary and respiratory excretion was somewhat lower in the high dose study compared to the low dose study, thereby suggesting that saturation of absorption cannot be excluded at the 45 mg/kg bw/d dose level.

Excretion

Minipigs: Radiolabelled substance was rapidly excreted in miniature swine via faeces (59% within 48 h) and urine (14% within 24 h). Pre-treatment with 10 daily 5 mg radiolabelled daminozide/kg bw doses did not importantly change the excretion data.

Rats: A single oral dose of 1 mg radiolabelled daminozide/kg bw to rats was excreted via urine (47%), faeces (32%) and the lungs ($^{14}CO_2$: 7%; some volatile compounds in expired air: <1%). The oral dose of 1.21 mg of radiolabelled daminozide to rats was rapidly eliminated (nearly completely within 48 h) via faeces (70%), urine (24%) and lungs (2.4%). In total, more than 96% of the administered radiolabelled substance was excreted at the time of sacrifice.

Table 2.6.1.1-1: Excretion of daminozide and UDMH in urine and faeces of rats (% of the administered dose); *Anonymous (1993)*

Urine			**Faeces**		
	UDMH	**Daminozide**		**UDMH**	**Daminozide**
0 - 6 h	0.55	6.61			
6 - 12 h	**8.14**	3.63	0 - 12 h	0.17	8.5
12 - 24 h	**17.4**	2.56	12 - 24 h	0.87	18
24 - 48 h	**2.69**	0.58			
Cumulative	28.7	13.4	Cumulative	1	27

Distribution

Minipigs: In minipigs receiving a single 5 mg/kg dose, radiolabel was recovered mainly from liver (0.043 mg eq/kg). Only in the kidneys were increased levels of radioactivity found in pre-treated animals (10 daily doses of 5 mg/kg/d of unlabelled material following a dose of 5 mg/kg of radiolabelled material) compared to animals that had not been pre-

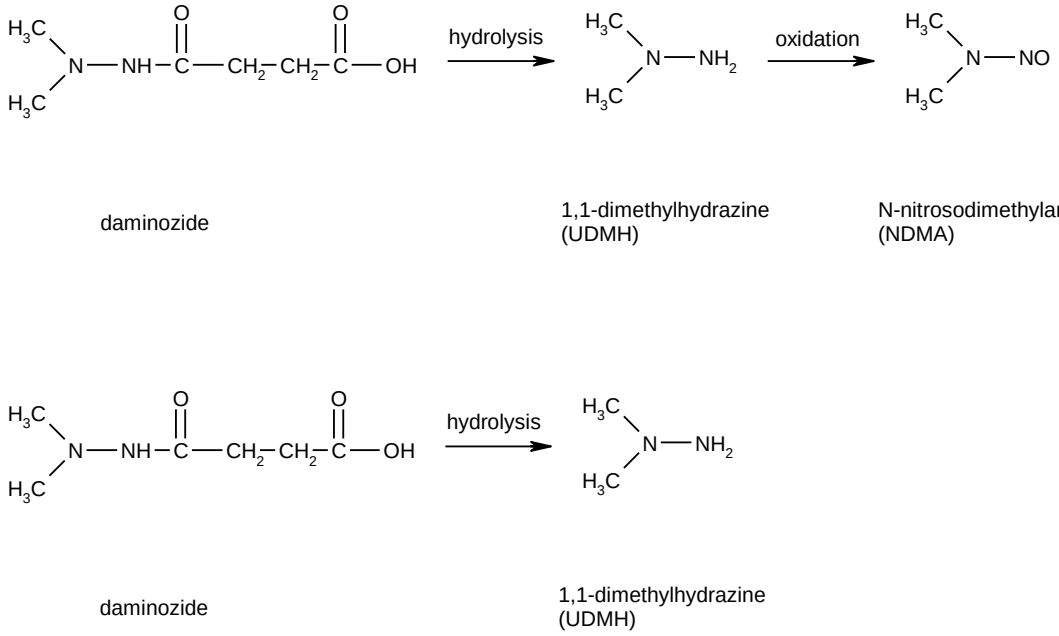
treated (0.048 compared to 0.020 mg eq/kg).

Rats: Some organs and tissues were examined for radioactivity upon administration of a single oral dose of 1 mg radiolabelled daminozide/kg bw to male rats. Liver, blood, and lungs contained 0.045, 0.020, and 0.044 mg eq/kg, respectively (0.18%, 0.10%, and 0.03% of the administered dose, respectively). Taking the inter-laboratory and interspecies variation into account, tissue levels are in similar ranges. Comparison of tissue levels in the Fisher 344 rat studies at 96 h reveals that relative to dose, tissue residues are smaller at the high doses compared to low doses (45 versus 1 mg/kg bw, respectively). This is likely to be due to saturation in absorption. It should be noted that a rather long terminal half-life was observed in the second rat study, indicating tissue retention of radiolabelled substance.

Metabolism

In miniature swine, daminozide was converted to unsymmetrical dimethyl hydrazine **(**UDMH) and N-nitrosodimethylamine (NDMA), as both compounds were reported to be found in urine and faeces. UDMH was also found in liver. In rats that received a single oral dose of 1 mg test substance/kg bw, UDMH was found as an important metabolite in urine and faeces (accounting for 29% and 1% of the administered dose, respectively). Unchanged test substance accounted for 13 and 27% of the administered dose in urine and faeces, respectively. Further comparison between species was limited because of the differences in the dose level at which metabolism was investigated in the rat and minipig.

Figure 2.6.1.1-1: Proposed metabolism of daminozide



In vitro comparative study (*Anonymous, 2017*): [^{14}C]-Daminozide (5 and 50 µM; purity: >98%) was incubated with mouse, rat, dog or human hepatocytes for 0.5, 1 and 3 hours. Incubations in the absence of hepatocytes were also

conducted at both [^{14}C]-daminozide concentrations for 3 hours. Samples incubated with 7-ethoxy[3-^{14}C]coumarin (7EC) were used as a positive control. The initial hepatocyte viability was determined by the trypan blue exclusion test. Incubation samples were analysed by HPLC with both off-line (5 µM [^{14}C]-daminozide samples) and on-line (50 µM [^{14}C]-daminozide samples) radioactive monitoring. The proportions of produced metabolites and parent [^{14}C]-daminozide were quantified. Selected samples were analysed by LC-MS with the aim of identifying the metabolites produced. [^{14}C]-Daminozide (5 and 50 µM) remained as unchanged parent compound following incubation with mouse, rat, dog and human hepatocytes for 0, 0.5, 1 and 3 hours. Only one metabolite (UDMH) was detected by radio-HPLC, but at levels below the limit of quantification (<1%) in all species, in the treated as well as control samples (in the absence of hepatocytes). Using LC-MS/MS, only NDMA was detected in mouse, rat and human treated as well as non-hepatocyte control samples.

Table 2.6.1.1-2: Parent compound: Total % of radioactivity (mean value of duplicates)

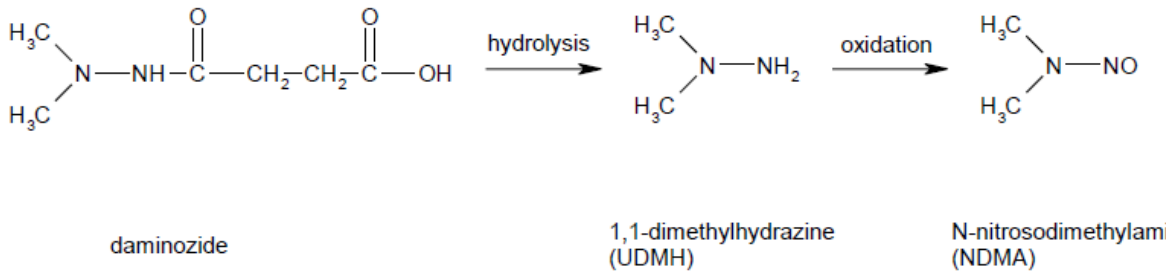
[^{14}C]-Daminozide concentration	**5µM**				**50µM**			
Incubation time [hours]	**0**	**0.5**	**1**	**3**	**0**	**0.5**	**1**	**3**
Mouse hepatocytes	96.9	97.4	97.4	97.4	91.8	95.7	91.5	93.4
Rat hepatocytes	97.2	97.2	97.1	96.9	95.8	98.7	97.0	97.0
Dog hepatocytes	97.3	97.3	97.1	97.2	97.9	97.6	96.6	96.5
Human hepatocytes	96.8	97.0	97.4	97.8	95.1	96.0	93.7	97.9

In general, the presence of metabolites in the control samples without hepatocytes (not only in the treated ones) might indicate that the parent compound is rather degraded than metabolised by hepatocyte enzymes. However, in case of daminozide, the major metabolite (UDMH) was detected in samples at low levels (<1%, i.e. below the limit of quantification by radio-HPLC), which could be explained by too short incubation (maximally 3 hours) of hepatocytes with the test substance. This conclusion is supported by the results of the toxicokinetic study (*Anonymous, 1993*) showing that UDMH represented the predominant compound in urine within time intervals 6 – 12 and 12 – 24 hours after the daminozide administration (*see Table 2.6.1.1-1*). In addition, the hydrolysis in aqueous solution from the parent molecule to UDMH is characterized by the maximum hydrolytic conversion between 4 – 24 hours (*Connor, 2012*). Using hepatocytes in *in vitro* comparative metabolism study, 2 – 4 hour incubation is generally recommended. However, longer incubation times can be used dependent on the model system (e.g. plated hepatocytes) or testing laboratory protocol. RMS is of the opinion that *in vitro* comparative metabolism study did not fulfil its purpose regarding the role of UDMH and NDMA in human metabolism, which was caused by inappropriate duration of the incubation of hepatocytes with the test substance..

RAC general comment

Daminozide is a plant growth regulator reducing internode length and promoting flower production by the inhibition of gibberellins and ethylene. *In vivo*, daminozide is metabolized to 1,1-dimethylhydrazine (unsymmetrical dimethyl hydrazine, UDMH) and N-nitrosodimethylamine (NDMA). Rats treated with a single oral dose of 1 mg/kg bw daminozide excreted within 48 hours 29% and 1% of the administered dose as UDMH in urine and faeces, respectively (Anonymous, 1993). The fraction of unchanged parent substance excreted in urine and faeces accounted for 13% and 27%, respectively. Earlier studies in rats showed an oral absorption rates between 55% and 28% after single doses of 1 or 5.7 mg/kg bw, respectively. Newer data considering both urinary and respiratory excretion after single dose of 45 mg/kg bw daminozide points to an oral absorption value of 35% (Anonymous, 1999).

Figure: Proposed metabolism of daminozide leading to the potentially toxic metabolites UDMH and NDMA.



An *in vitro* comparative metabolism study in rat, mouse, dog and human hepatocytes was conducted by Anonymous (2017). [^{14}C]-daminozide (purity > 98%) at dose levels of 0, 5 and 50 µM was incubated with hepatocytes for 0.5, 1 or 3 hours, and the amounts of produced metabolites and parent chemical were quantified. Samples incubated with 7-ethoxy[3-^{14}C]coumarin were used as a positive control. The results from the study showed that daminozide remained essentially unchanged after incubation for up to 3 hours with each of the mouse, rat, dog and human hepatocytes. No specific differences in metabolite profiles were detected between the species, and the low quantities of UDMH and NDMA detected in samples at concentrations below the LOQ may possibly be a consequence of degradation rather than to metabolism. Considering that biotransformation of daminozide was demonstrated *in vivo*, it is not clear why *in vitro* incubations failed to produce any significant amount of both metabolites. Short incubation time and low cells viability/enzyme expression are some of the possible limitations of the study discussed by DS. There is no *in vivo* human data on metabolism, however it can reasonably be assumed that biotransformation of daminozide to UDMH and NDMA occurs also in humans.

In Annex VI of the CLP Regulation, UDMH (Index No: 007-012-00-5) is classified for human health hazards as Carc. 1B; H350, Acute Tox. 3*; H331, Acute Tox. 3*; H301, and Skin Corr. 1B; H314. NDMA (Index No: 612-077-00-3) is listed as Carc. 1B; H350 (C≥0.001%), Acute Tox. 2*; H330, Acute Tox. 3*; H301, and STOT RE 1; H372**.

2.6.2 Summary of acute toxicity

Based on the results of acute toxicity studies, daminozide does not need to be classified according to Regulation (EC) No 1272/2008 as amended for acute oral, dermal, respiratory toxicity, skin or eye irritation and skin sensitization. The results of the acute toxicity studies, the irritation studies, and the sensitization studies that are suitable for evaluation in the context of AIR III renewal are presented below in tabular format.

2.6.2.1 Acute toxicity - oral route [equivalent to section 10.1 of the CLH report template]

Table 7: Summary table of animal studies on acute oral toxicity

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance**	**Dose levels, duration of exposure**	**Value LD_{50}**	**Reference**
Acute oral toxicity OECD TG 401 **Acceptable study**	Rat (Wistar Albino) 5animals/sex	Test material: Alar Purity: 99.42%	A single dose of 5000 mg/kg bw (by gavage) Rats were observed 1, 2, 4 hours post dose and once daily for 14 days	LD_{50} > 5000 mg/kg bw (male/female)	*Anonymous (1994a)*

Table 8: Summary table of human data on acute oral toxicity

Type of data/report	**Test substance**	**Relevant information about the study (as applicable)**	**Observations**	**Reference**	**Type of data/report**
No data available					

Table 9: Summary table of other studies relevant for acute oral toxicity

Type of data/report	**Test substance**	**Relevant information about the study (as applicable)**	**Observations**	**Reference**	**Type of data/report**
No data available					

2.6.2.1.1 Short summary and overall relevance of the provided information on acute oral toxicity

A study was performed to investigate the acute oral toxicity of the test material in rats in line with standardised guideline OECD 401. The rats were dosed via gavage and were sacrificed at the end of the observation period. No animal died during the exposure and 14-day post exposure period and no abnormalities were observed at necropsy. Only instances of soiling of the anogenital area were noted during the observation period. Body weight gains were normal. Under the conditions of this study the LD_{50} was found to be greater than 5000 mg/kg bw.

2.6.2.1.2 Comparison with the CLP criteria regarding acute oral toxicity

According to CLP criteria (Regulation (EC) No. 1272/2008), the last acute toxicity hazard category (Category 4) for the oral exposure is characterized by the following value of LD_{50}: $300 < LD_{50} \leq 2000$ mg/kg bw. Based on the acute oral toxicity study, LD_{50} for daminozide is higher than 5000 mg/kg bw.

2.6.2.1.3 Conclusion on classification and labelling for acute oral toxicity

As LD_{50} is higher than 5000 mg/kg bw, classification according CLP criteria (Regulation (EC) No. 1272/2008) is not required.

2.6.2.2 Acute toxicity - dermal route [equivalent to section 10.2 of the CLH report template]

Table 10: Summary table of animal studies on acute dermal toxicity

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance**	**Dose levels, duration of exposure**	**Value LD_{50}**	**Reference**
Acute dermal toxicity OECD TG 402 **Acceptable study**	Rabbit (New Zealand Albino) 5 animals/sex	Test material: Alar Purity: 99.42%	A single dose of 5000 mg/kg bw applied for 24 hours Dermal response was recorded on days 1, 7, and 14 Rabbits were observed 1, 2, 4 hours post dose and once daily for 14 days	LD_{50} > 5000 mg/kg bw (male/female)	*Anonymous (1994b)*

Table 11: Summary table of human data on acute dermal toxicity

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference	Type of data/report
No data available					

Table 12: Summary table of other studies relevant for acute dermal toxicity

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference	Type of data/report
No data available					

2.6.2.2.1 Short summary and overall relevance of the provided information on acute dermal toxicity

A study was performed to investigate the acute dermal toxicity of the test material in rabbits in accordance with the standardised guideline OECD 402. One animal died on day 13 with no pre-death physical signs. Clinical signs noted included diarrhoea, soiling of the anogenital area, emaciation and few faeces. Erythema and oedema, absent to well defined on day 1, were absent on days 7 and 14. Body weight gains were normal and no abnormalities were observed in the survivors at necropsy. The necropsy observations of the dead animal are consistent with pulmonary infection and are not considered to be related to the substance. Under the conditions of this study the LD_{50} was found to be greater than 5000 mg/kg bw.

2.6.2.2.2 Comparison with the CLP criteria regarding acute dermal toxicity

According to CLP criteria (Regulation (EC) No. 1272/2008), the last acute toxicity hazard category (Category 4) for the dermal exposure is characterized by the following value of LD_{50}: $1000 < LD_{50} \leq 2000$ mg/kg bw. Based on the acute dermal toxicity study, LD_{50} for daminozide is higher than 5000 mg/kg bw.

2.6.2.2.3 Conclusion on classification and labelling for acute dermal toxicity

As LD_{50} is higher than 5000 mg/kg bw, classification according CLP criteria (Regulation (EC) No.1272/2008) is not required.

2.6.2.3 Acute toxicity - inhalation route [equivalent to section 10.3 of the CLH report template]

Table 13: Summary table of animal studies on acute inhalation toxicity

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance, form and particle size (MMAD)**	**Dose levels, duration of exposure**	**Value LD_{50}**	**Reference**
Acute inhalation toxicity Nose-only exposure OECD TG 403 Deviations: MMAD = 6.7 µm (i.e. > recommended 4 µm) **Study limited due to the value of MMAD**	Rat (Sprague-Dawley) 5 animals/sex	Test material: Alar Purity: 99.42% MMAD = 6.7 µm with an average GSD = 2.8	2.1 mg/l (average concentration; range: 1.2-3.2 mg/l) Duration of exposure: 4 hours Rats were observed for 14 days post exposure	LC_{50} > 2.1 mg/l (male/female; the highest attainable dose)	*Anonymous (1994a)*

Table 14: Summary table of human data on acute inhalation toxicity

Type of data/report	**Test substance**	**Relevant information about the study (as applicable)**	**Observations**	**Reference**	**Type of data/report**
No data available					

Table 15: Summary table of other studies relevant for acute inhalation toxicity

Type of data/report	**Test substance**	**Relevant information about the study (as applicable)**	**Observations**	**Reference**	**Type of data/report**
No data available					

2.6.2.3.1 Short summary and overall relevance of the provided information on acute inhalation toxicity

A study was performed to investigate the acute inhalation toxicity of the test material in rats in accordance with the standardised guideline OECD 403. The test material was milled before use. The highest attainable test concentration was 2.1 mg/L. According to OECD TG 403, MMAD should range from 1 – 4 µm in order to ensure the exposure of all relevant regions of the respiratory tract. This recommendation cannot be, however, achieved for all substances. The study is limited by MMAD = 6.7 µm (GSD = 2.8); no justification of MMAD exceeding 4 µm was provided by the applicant. No animals died during the exposure or 14-days post exposure observation period. However, during the 2 to 4 hour post-exposure period and during the 14-day observation period a few scattered signs of nasal discharge were observed. Normal body weight gain was noted. There were no abnormalities observed at necropsy. Under the conditions of this study the LC_{50} was found to be greater than 2.1 mg/L.

2.6.2.3.2 Comparison with the CLP criteria regarding acute inhalation toxicity

According to CLP criteria (Regulation (EC) No. 1272/2008), the last acute toxicity hazard category (Category 4) for the inhalation exposure (dust and mists) is characterized by the following value of LC_{50}: $1 < LC_{50} \leq 5$ mg/l. Based on the acute inhalation toxicity study, LC_{50} for daminozide is higher than the highest practically attainable concentration 2.1 mg/l.

2.6.2.3.3 Conclusion on classification and labelling for acute inhalation toxicity

As LC_{50} is higher than 2.1 mg/l, representing the highest achievable concentration, classification according to CLP criteria (Regulation (EC) No. 1272/2008) is not required.

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

Acute toxicity studies according to OECD TG 401, 402, and 403 are provided in this dossier. The DS proposed no classification for oral, dermal and inhalation acute toxicity as the CLP criteria were not met.

Comments received during public consultation

During the public consultation, two Member State Competent Authorities (MSCAs) and one manufacturer supported no classification for acute toxicity via oral, dermal, and inhalation route.

Assessment and comparison with the classification criteria

Acute oral toxicity

One GLP and guideline compliant study according to OECD TG 401 is available (Anonymous, 1994a). Wistar rats (5/dose/sex) were exposed via gavage to a single dose of 5000 mg/kg bw daminozide technical (purity 99.42%) and sacrificed at the end of the 14 days observation period. No deaths were reported, and no abnormalities were observed at necropsy. Under the

conditions of this study the LD_{50} was greater than 5000 mg/kg bw and thus above the acute toxicity estimates (ATE) for acute toxicity hazard by the oral route for category 4 (300<ATE≤2000). The study is considered acceptable and **RAC concludes that classification of daminozide for acute oral toxicity is not warranted.**

Acute dermal toxicity

One GLP and guideline compliant study according to OECD TG 402 is available (Anonymous, 1994b). New Zealand white (NZW) rabbits (5/dose/sex) were exposed to daminozide technical (purity 99.42%) at 5000 mg/kg bw via dermal application of the moistened with distilled water substance. One animal died on day 13. Necropsy examination indicated signs consistent with pulmonary infection that are not considered to be related to the substance. Clinical signs noted included diarrhoea, soiling of the anogenital area, emaciation and few faeces. Under the conditions of this study the LD_{50} was greater than 5000 mg/kg bw, and thus above the acute toxicity estimates (ATE) for category 4 (1000 mg/kg bw<ATE≤2 000 mg/kg bw). The study is considered acceptable and RAC concludes that **classification of daminozide for acute dermal toxicity is not warranted**.

Acute inhalation toxicity

A GLP and guideline compliant acute inhalation toxicity study equivalent to OECD TG 403 is available (Anonymous, 1994a). The test substance was milled before use and Sprague-Dawley rats (5/sex/dose) were exposed nose-only to daminozide technical (purity 99.42%) at the highest attainable test concentration of 2.1 mg/L. As a deviation from OECD TG 403, particle size exceeded the recommended size range of 1-4 µm (MMAD=6.7, GSD=2.8) was reported. No deaths were reported, and no abnormalities were observed at necropsy. Clinical observation included a few scattered signs of nasal discharge during the 14-day observation period. Under these study conditions, the LC_{50} was greater than 2.1 mg/L. Since no higher dust aerosol concentrations of daminozide could be generated, and no deaths or clinical signs of toxicological importance were demonstrated at the highest tested dose, the classification criteria for acute inhalation toxicity are not met. Despite the higher MMAD of 6.7 µm, the study is considered acceptable and RAC concludes that **classification of daminozide for acute inhalation toxicity is not warranted.**

2.6.2.4 Skin corrosion/irritation [equivalent to section 10.4 of the CLH report template]

Table 16: Summary table of animal studies on skin corrosion/irritation

Method, guideline, deviations[1] if any	**Species, strain, sex, no/group**	**Test substance**	**Dose levels, duration of exposure**	**Results** **- Observations and time point of onset[2]** **- Mean scores/animal** **- Reversibility**	**Reference**
Acute dermal irritation OECD TG 404 **Acceptable study**	Rabbit (New Zealand White) 5 ♂, 1♀	Test material: Alar Purity: 99.42% Form: powder	0.5 g for 4 hours Dermal reactions were scored at 30, 60 minutes, 24, 48 and 72 hours	Erythema and oedema: absent to slight at 30 to 60 minutes after patch removal, absent at 24, 48, and 72 hours Mean score/animal = 0 (erythema/eschar as well as oedema at 24, 48, and 72 hours after patch removal) No abnormal physical signs were noted (ulceration, necrosis, tissue destruction, changes in general health)	*Anonymous (1994)*

Table 17: Summary table of human data on skin corrosion/irritation

Type of data/report	**Test substance**	**Relevant information about the study (as applicable)**	**Observations**	**Reference**	**Type of data/report**
No data available					

Table 18: Summary table of other studies relevant for skin corrosion/irritation

Type of data/report	**Test substance**	**Relevant information about the study (as applicable)**	**Observations**	**Reference**	**Type of data/report**
No data available					

2.6.2.4.1 Short summary and overall relevance of the provided information on skin corrosion/irritation

A study was performed to assess the irritative potential of the test material in accordance with the standardised guideline OECD 404. The test material (0.5g) was applied to the skin of albino rabbits for 4 hours. The dermal reactions were scored at 30, 60 minutes, 24, 48 and 72 hours according to the Draize scoring system. Erythema and oedema,

absent to slight at 30 to 60 minutes after patch removal, were absent at 24, 48, and 72 hours. Mean score/animal was 0 for erythema/eschar as well as oedema at 24, 48, and 72 hours after patch removal. Under the conditions of the test, the test material was not found to be irritating to the rabbit skin.

Table 2.6.2.4.1-1: Draize scores in rabbits from the acute dermal irritation study

Scores observed after	30-60 minutes	24 hours	48 hours	72 hours
Erythema	0, 1, 0, 0, 0, 0	0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0
Oedema	0, 1, 0, 0, 0, 0	0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0

2.6.2.4.2 Comparison with the CLP criteria regarding skin corrosion/irritation

Using the results of animal testing, an active substance is classified as the irritant (Category 2) according to CPL criteria (Regulation (EC) No. 1272/2008), if the mean score for erythema/eschar or oedema ≥ 2.3 and ≤ 4 in at least 2 of 3 tested animals (calculated from scores at 24, 48, 72 hours after the patch removal). In the study with daminozide, the mean score = 0 for erythema/eschar as well as oedema in all tested animals.

2.6.2.4.3 Conclusion on classification and labelling for skin corrosion/irritation

As the mean score for erythema/eschar as well as oedema = 0 in all tested animals at 24, 48, and 72 hours after the patch removal, the classification according to CLP criteria (Regulation (EC) No. 1272/2008) is not required.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

No classification for skin corrosion/irritation was proposed by the DS because no dermal reactions meeting the classification criteria were reported in the available guideline study of daminozide.

Comments received during public consultation

During the public consultation, two MSCAs and one manufacturer supported no classification for this endpoint.

Assessment and comparison with the classification criteria

An Acute Dermal Irritation/Corrosion study according to GLP and OECD TG 404 was performed with daminozide technical (purity 99.42%; Anonymous, 1994). The test substance (0.5 g) was applied to the skin of NZW rabbits (5 ♂, 1♀) for 4 hours, and dermal reactions were assessed according to the Draize scoring system. Slight to absent erythema and oedema in 1/6 animals were reported at 30 to 60 minutes after patch removal, and were absent at 24, 48, and 72 hours. Mean scores for erythema/eschar and oedema were zero, and therefore, under the conditions of the test, the test material was not irritating to the rabbit skin. The study is

considered acceptable and RAC agrees with the DS that CLP classification criteria for skin corrosion/irritation are not fulfilled and **classification of daminozide for this endpoint is not warranted.**

2.6.2.5 Serious eye damage/eye irritation [equivalent to section 10.5 of the CLH report template]

Table 19: Summary table of animal studies on serious eye damage/eye irritation

Method, guideline, deviations[1] if any	**Species, strain, sex, no/group**	**Test substance**	**Dose levels duration of exposure**	**Results - Observations and time point of onset[2] - Mean scores/animal - Reversibility**	**Reference**
Acute eye irritation OECD TG 405 **Acceptable study**	Rabbit (New Zealand Albino) 6 animals (4 ♂, 2 ♀)	Test material: Alar Purity: 99.42% Form: powder	0.1 g. placed into conjunctival sac Ocular responses were recorded at 1 hour, on day 1, 2, 3 and 7 after exposure	Corneal opacity: none at any observation period; mean score/animal = 0 (max. possible = 4) Iritis: 1/6 animals, cleared by day 2; mean score/animal = 0.06 (max. possible = 2) Conjunctival redness: 6/6 animals, cleared by day 7; mean score/animal = 1.55 (max. possible = 3) Conjunctival chemosis: 6/6 animals, cleared by day 7; mean score/animal = 1.62 (max. possible = 4) All ocular abnormalities resolved on day 7	*Anonymous (1994c)*

Table 20: Summary table of human data on serious eye damage/eye irritation

Type of data/report	**Test substance**	**Relevant information about the study (as applicable)**	**Observations**	**Reference**	**Type of data/report**
No data available					

Table 21: Summary table of other studies relevant for serious eye damage/eye irritation

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference	Type of data/report
No data available					

2.6.2.5.1 Short summary and overall relevance of the provided information on serious eye damage/eye irritation

The irritative potential of the test material was investigated in a study conducted in accordance with the standardised guideline OECD 405. The test material was applied into conjunctival sac of one eye of each albino rabbit. The contralateral eye served as a control. Ocular responses were recorded at 1 hour, on day 1, 2, 3 and 7. Fluorescein was used to determine corneal effects on day 1. The Draize scoring system was applied to assess the irritancy. Under the conditions of this study the test material was found to be mildly irritating to the rabbit eye. Iritis and conjunctival irritation was cleared within 7 days.

Table 2.6.2.5.1-1: Draize scores in rabbits from the acute eye irritation study

Scores observed after	1 hour	24 hours	48 hours	72 hours	7 days	Mean score[b]
Corneal opacity	0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0
Iritis	0, 0, 0, 0, 0, 0	0, 1, 0, 0, 0, 0	0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0
Conjunctival redness	1, 1, 1, 1, 1, 1	1, 2, 2, 2, 1, 2	1, 2, 2, 1, 2, 1	1, 2, 2, 1, 2, 1	0, 0, 0, 0, 0, 0	1, 2, 2, 1.3, 1.7, 1.3
Conjunctival chemosis (oedema)	2, 2, 2, 2, 2, 2	2, 2, 2, 2, 2, 2	2, 2, 2, 1, 2, 2	1, 2, 2, 0, 1, 0	0, 0, 0, 0, 0, 0	1.7, 2, 2, 1, 1.7, 1.3
Conjunctival discharge	2, 2, 2[a], 2, 2, 2	2, 2, 1 ,1, 2, 2	0, 2, 1, 1, 2, 2	0, 1, 0, 0, 0, 0	0, 0, 0, 0, 0, 0	0.7, 1.7, 0.7, 0.7, 1.3, 1.3

[a] test article remaining in conjunctiva

[b] calculated from scores at 24, 48, 72 hours

2.6.2.5.2 Comparison with the CLP criteria regarding serious eye damage/eye irritation

According to CPL criteria (Regulation (EC) No. 1272/2008), an active substance is considered to be irritating to eyes (Category 2), if the following positive response (calculated as the mean scores following grading at 24, 48, and 72 hours after the instillation) is observed at least in 2 of 3 tested animals: corneal opacity ≥ 1 and/or iritis ≥ 1; and/or conjunctival redness ≥ 2 and/or conjunctival chemosis (oedema) ≥ 2. The positive reaction fully reverses within an observation period of 21 days. In the study with daminozide, the mean score for conjunctival redness as well as chemosis = 2 in 2 animals. However, 6 animals instead of 3 were tested.

2.6.2.5.3 Conclusion on classification and labelling for serious eye damage/eye irritation

The classification according CLP criteria (Regulation (EC) No. 1272/2008) is not required since the values of mean scores sufficient to trigger the classification were reached only in 2 from 6 animals.

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

The DS proposed no classification of daminozide since the mean scores for positive reactions sufficient to trigger the classification were reached only in 2 out of 6 animals and they were fully reversible within 7 days.

Comments received during public consultation

During the public consultation, two MSCAs and one manufacturer supported no classification for this endpoint.

Assessment and comparison with the classification criteria

In an Acute Eye Irritation/Corrosion study according to GLP and OECD TG 405, six NZW rabbits (4 ♂, 2 ♀) were subject to single ocular instillation of daminozide technical (purity 99.42%) into the one eye while the contralateral eye remained untreated and served as control (Anonymous, 1994c). Ocular responses were examined one hour after the treatment and on days 1, 2, 3 and 7. The results from the test showed iritis in 1/6 animals with mean score/animal of 0.06 (max. possible=2), conjunctival redness in 6/6 animals with a mean score/animal of 1.55 (max. possible=3) and conjunctival chemosis in 6/6 animals with mean score/animal of 1.62 (max. possible=4). All ocular abnormalities resolved on day 7. Under the conditions of this study, daminozide was found to be mildly irritating to the rabbit eye.

***Table**: Ocular responses NZW rabbits (Anonymous, 1994c)*

Scores at	1 hour	24 hours	48 hours	72 hours	7 days	Mean score
Corneal opacity	0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0
Iritis	0, 0, 0, 0, 0, 0	0, 1, 0, 0, 0, 0	0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0	0, 0, 0, 0, 0, 0
Conjunctival redness	1, 1, 1, 1, 1, 1	1, 2, 2, 2, 1, 2	1, 2, 2, 1, 2, 1	1, 2, 2, 1, 2, 1	0, 0, 0, 0, 0, 0	1, 2, 2, 1.3, 1.7, 1.3
Conjunctival chemosis	2, 2, 2, 2, 2, 2	2, 2, 2, 2, 2, 2	2, 2, 2, 1, 2, 2	1, 2, 2, 0, 1, 0	0, 0, 0, 0, 0, 0	1.7, 2, 2, 1, 1.7, 1.3
Conjunctival discharge	2, 2, 2, 2, 2, 2	2, 2, 1 ,1, 2, 2	0, 2, 1, 1, 2, 2	0, 1, 0, 0, 0, 0	0, 0, 0, 0, 0, 0	0.7, 1.7, 0.7, 0.7, 1.3, 1.3

In the case of tests with six rabbits as used in the current study, the CLP Guidance states that category 2 is warranted if at least 4 out of 6 animals show mean scores per animal for corneal

opacity ≥1 and/or iritis ≥1; and/or conjunctival redness ≥2 and/or conjunctival chemosis (oedema) ≥2. The study is considered acceptable, and RAC agrees with the assessment of the DS that the mean scores sufficient to trigger the classification were reached only in 2 out of 6 animals and concludes that classification for serious eye damage/irritation is not warranted.

2.6.2.6 Respiratory sensitisation [equivalent to section 10.6 of the CLH report template]

Table 22: Summary table of animal studies on respiratory sensitisation

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference	Type of data/report
No data available					

Table 23: Summary table of human data on respiratory sensitisation

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference	Type of data/report
No data available					

Table 24: Summary table of other studies relevant for respiratory sensitisation

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference	Type of data/report
No data available					

2.6.2.6.1 Short summary and overall relevance of the provided information on respiratory sensitisation

Respiratory sensitizer is defined as a substance which causes hypersensitivity of the airways, when inhaled. No study with daminozide on respiratory sensitization is available. In addition, based on the results of acute inhalation study in rats (*see 2.6.2.3*), and Buehler test as well as local lymph node assay (*see 2.6.2.7*), daminozide is neither toxic via inhalation route nor skin sensitizer.

2.6.2.6.2 Comparison with the CLP criteria regarding respiratory sensitisation

The substance does not meet CLP criteria (Regulation (EC) No. 1272/2008) for classification of respiratory sensitisation.

2.6.2.6.3 Conclusion on classification and labelling for respiratory sensitisation

No classification is proposed

RAC evaluation of respiratory sensitisation

Summary of the Dossier Submitter's proposal

There is no data on respiratory sensitisation available for daminozide.

Comments received during public consultation

During the public consultation, one MSCA and one manufacturer supported no classification for this endpoint.

Assessment and comparison with the classification criteria

RAC was **unable to evaluate daminozide for respiratory sensitization due to lack of data.**

2.6.2.7 Skin sensitisation [equivalent to section 10.7 of the CLH report template]

Table 25: Summary table of animal studies on skin sensitisation

Method, guideline, deviations[1] if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Results	Reference
Local lymph node assay OECD TG 429 EPA OPPTS 870.2600 **Acceptable study**	Mice (CBA/CA) 5♂/group	Test material: daminozide Purity: 99.7% Form: powder	5, 10, 25% daminozide in DMSO Negative control: vehicle (DMSO) Positive control: α-hexylcinnamaldehyde	Stimulation index (SI): 5% test material = 0.58; 10% = 0.80; 25% = 1.28 (i.e. SI < 3, non-sensitizer) No mortality, clinical signs of systemic effect, effect on body weight Application site without irritation Greasy appearance of the fur of head and/or neck in all test groups on day 1 Positive control: valid; SI=6.34	*Anonymous (2003)*
Buehler test OECD TG 406 **Acceptable study**	Guinea pig (Dunkin Hartley) 10 ♂, 10 ♀	Test material: Alar Purity: 99.4% Form: powder	Induction: 100% Alar moistened with 0.3 ml of 0.9% saline for 6 hours (3 times repeated) Challenge: 14 days after induction in the same manner (once) Positive control: DNCB (dinitrochlorobenzene) in ethanol (induction) or acetone (challenge) Negative control: saline	No dermal response at challenge No mortality and effect on body weight Positive control: valid, i.e. all 10 animals exhibited clear dermal responses at challenge	*Anonymous (1994b*)

Table 26: Summary table of human data on skin sensitisation

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference	Type of data/report
No data available					

Table 27: Summary table of other studies relevant for skin sensitisation

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference	Type of data/report
No data available					

2.6.2.7.1 Short summary and overall relevance of the provided information on skin sensitisation

The potential of the test substance to cause skin sensitisation was evaluated in two studies: the Local lymph node assay (LLNA) and Buehler test.

In LLNA *(Anonymous, 2003)*, mice were subjected to topical applications of vehicle control (DMSO), positive control (α-hexylcinnamaldehyde) or one of the test formulations (5, 10 or 25% in DMSO) to the outer aspect of the auditory pinnae on days 1, 2 and 3. On day 6, titrated thymidine was injected intravenously into each animal. Five hours later the auricular lymph nodes were recovered and processed through a scintillation counter. The results are expressed as Stimulation Indices (SIs). In all groups treated with daminozide SI < 3, whereas in positive control SI = 6.34. The irritation was noted on the ears of the positive control animals on days 3 and 4. A greasy appearance of the fur of the head and/or neck was noted in all test animals on day 1 and in all positive controls throughout the observation period.

In Buehler test *(Anonymous, 1994b)* based on a range-finding study, a concentration of 100% test material moistened with saline was used for induction (3 exposures for 6 hours) and challenge (1 exposure for 6 hours, 14 days after the last induction). All animals treated with the test material showed no dermal response at challenge. All animals treated with positive control (dinitrochlorobenzene) exhibited clear dermal responses at challenge which were of greater incidence and severity than responses seen in the irritation control group (treated only at challenge).

Based on the results of these studies, the test material is not considered to be the skin sensitizer.

Table 2.6.2.7-1: Stimulation Index from the local lymph node assay

Concentration	Stimulation Index
5% daminozide	0.58
10% daminozide	0.80
25% daminozide	1.28
25% α-hexylcinnamaldehyde	6.34

2.6.2.7.2 Comparison with the CLP criteria regarding skin sensitisation

According to CPL criteria (Regulation (EC) No. 1272/2008), an active substance is considered to be the skin sensitizer (Subcategory 1B) based on the results of Buehler and local lymph node assay if (i) ≥ 15% of animals respond at > 20% topical induction dose in Buehler assay; or if (ii) EC3 (estimated concentration needed to produce a stimulation index of 3) > 2% in the local lymph node assay. In the study with daminozide, no animal responded in Buehler test and SI = 1.28 at the highest concentration (25%) of the test substance in the local lymph node assay.

2.6.2.7.3 Conclusion on classification and labelling for skin sensitisation

As Buehler test and local lymph node assay with daminozide were negative, the classification according CLP criteria (Regulation (EC) No. 1272/2008) is not required.

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

Based on negative results from one Buehler test in Guinea pigs with alar (purity 99.4%) and one Local Lymph Node Assay (LLNA) test in mice with daminozide (purity 99.7%), the DS proposed no classification for skin sensitisation.

Comments received during public consultation

One MSCA and one manufacturer supported no classification for this endpoint. One MSCA disagreed with the overall conclusion that both the Buehler and the LLNA tests are negative pointing out that concentrations only up to 25% daminozide were tested in the LLNA test, and the negative Buehler test is less sensitive than LLNA.

Assessment and comparison with the classification criteria

A LLNA and a Buehler test are available for assessment of the skin sensitisation potential of daminozide.

In a LLNA test (Anonymous, 2003), CBA/CA mice (5 ♂/dose group) were treated with applications of vehicle control (DMSO), positive control (α-hexylcinnamaldehyde) or daminozide technical test formulations of 5, 10 or 25% in DMSO according to the provisions of GLP and OECD TG 429. Greasy appearance of the fur of head and/or neck in all test groups was reported on day 1 of the test, however no signs of irritation on the application site of daminozide are documented. A stimulation index of SI<3 was calculated in all treatment groups for daminozide, whereas in positive control SI=6.34 (Table below).

***Table**: Stimulation indexes in the LLNA study (Anonymous, 2003)*

Concentration	Stimulation Index (SI)	Result
5% daminozide	0.58	negative
10% daminozide	0.80	negative
25% daminozide	1.28	negative
25% α-hexylcinnamaldehyde	6.34	positive

In a Buehler test with Guinea pigs (10 ♂, 10 ♀) according to OECD TG 406 (Anonymous, 1994b), a test concentration of 100% Alar® moistened with saline was used for induction (3 exposures for 6 hours) and challenge (1 exposure for 6 hours, 14 days after the last induction). A clear dermal response at challenge was observed only with the positive control (dinitrochlorobenzene in acetone), while animals treated with the test substance showed no reactions.

RAC further notes that the results from the LLNA test indicate a dose response with increasing test concentrations, and no specific justification for the selection of dose levels only up to 25% is provided. According to the report, the highest suitable dose was established in a preliminary screening test. Considering that the substance did not cause skin irritation up to 100%, RAC is

on the opinion that the study is inadequate for classification purposes.

Based on both negative tests, RAC supports the DS opinion that **classification of daminozide for skin sensitisation is not warranted.**

2.6.2.8 Phototoxicity

Table 28: Summary table of studies on phototoxicity

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference	Type of data/report
No data available. As daminozide was shown not to absorb electromagnetic radiation within the range of 290 – 700 nm, the study on phototoxicity is not required.					

Table 29: Summary table of human data on phototoxicity

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference	Type of data/report
No data available					

Table 30: Summary table of other studies relevant for phototoxicity

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference	Type of data/report
No data available					

2.6.2.9 Aspiration hazard [equivalent to section 10.13 of the CLH report template]

Table 31: Summary table of evidence for aspiration hazard

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference	Type of data/report
No data available					

2.6.2.9.1 Short summary and overall relevance of the provided information on aspiration hazard

No information on aspiration hazard relating daminozide is available.

2.6.2.9.2 Comparison with the CLP criteria regarding aspiration hazard

According to the CLP criteria (Regulation (EC) No. 1272/2008), an active substance is included in the hazard category (Category 1) for aspiration toxicity: (i) based on reliable and good quality human evidence or (ii) if it is a hydrocarbon and has a kinematic viscosity of 20.5 mm^2/s or less, measured at 40°C. The second criterion is related only to liquid

substances. As for Daminozide, it represents a solid active substance and no data on aspiration hazard in humans are available.

2.6.2.9.3 Conclusion on classification and labelling for aspiration hazard

The substance does not meet CLP criteria (Regulation (EC) No. 1272/2008) for aspiration hazard. No classification is proposed.

RAC evaluation of aspiration toxicity

Summary of the Dossier Submitter's proposal

Daminozide is a solid substance and no data on aspiration hazard in humans is available. The DS proposed no classification for aspiration hazard.

Comments received during public consultation

No comments on this endpoint were provided during public consultation.

Assessment and comparison with the classification criteria

According to the CLP Regulation, classification for aspiration hazard can be based on reliable and good quality human evidence or if the substance with a kinematic viscosity of 20.5 mm^2/s or less, measured at 40°C. Since none of the criteria is met, RAC agrees with DS that **no classification for aspiration toxicity is warranted.**

2.6.2.10 Specific target organ toxicity-single exposure (STOT SE) [equivalent to section 10.11 of the CLH report template]

Table 32: Summary table of animal studies on STOT SE (specific target organ toxicity-single exposure)

Method, guideline, deviations[1] if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results** **- NOAEL/LOAEL** **- target tissue/organ** **- critical effects at the LOAEL**	**Reference**
Acute oral toxicity OECD TG 401 Rat (Wistar Albino) 5animals/sex/dose **Acceptable study**	Test material: Alar Purity: 99.42% Oral route: by gavage A single dose of 5000 mg/kg bw	LD50 > 5000 mg/kg bw (male/female) No mortality Only instances of soiling of the anogenital area were observed during 14-day observation period No abnormalities at necropsy	*Anonymous (1994a)*
Acute inhalation toxicity OECD TG 403 Deviations: MMAD = 6.7 µm (> recommended 4 µm) Rat (Sprague-Dawley) 5animals/sex/dose **Study limited due to the value of MMAD**	Test material: Alar Dose level: 2.1 mg/l (average concentration; range: 1.2-3.2 mg/l) Purity: 99.42% Inhalation route: dust Nose-only exposure Duration of exposure: 4 hours	LC50 > 2.1 mg/l (male/female; the highest attainable dose) No mortality No abnormalities at necropsy A few scattered signs of nasal discharge were observed 2 – 4 hours post-exposure and during 14-day observation period	*Anonymous (1994a)*

Acute dermal toxicity OECD TG 402 Rabbit (New Zealand Albino) 5animals/sex/dose **Acceptable study**	Test material: Alar Purity: 99.42% Dermal route A single dose of 5000 mg/kg bw Duration of exposure: 24 hours	LD50 > 5000 mg/kg bw (male/female) One animal died from pulmonary infection No abnormalities in survivors at necropsy Clinical findings during 14-day observation period: diarrhoea, few faeces, soiling of the anogenital area, reduction in weight	*Anonymous (1994b)*
Acute neurotoxicity study OECD 424 Rats (Crl:CD(SD)) Females, Males 10 animals/group **Acceptable study**	Daminozide Oral route: by gavage; a single dose Dose levels: 0, 500, 1000, 2000 mg/kg bw/day Vehicle: 0.5% carboxymethylcellulose Purity: 100%	NOAEL: 1000 mg/kg bw/day LOAEL: 2000 mg/kg bw/day based on decreased locomotor activity (total distance, basic and fine movement) Except for decreased locomotor activity, no treatment-related clinical and FOB observations No lesions in neural tissues	*Anonymous (2012a*)

Table 33: Summary table of human data on STOT SE (specific target organ toxicity-single exposure)

Type of data/report	**Test substance**	**Relevant information about the study (as applicable)**	**Observations**	**Reference**	**Type of data/report**
No data available					

Table 34: Summary table of other studies relevant for STOT SE (specific target organ toxicity-single exposure)

Type of data/report	**Test substance**	**Relevant information about the study (as applicable)**	**Observations**	**Reference**	**Type of data/report**
No data available					

2.6.2.10.1 Short summary and overall relevance of the provided information on specific target organ toxicity – single exposure (STOT SE)

Acute toxicity studies are summarised in *2.6.2*. Except for one dead male of the acute dermal study, no mortality was observed. The necropsy of the dead animal revealed abnormalities of the lungs, pleural cavity, and wetness of the nose/mouth area, which was consistent with the pulmonary infection not related to the treatment. Other abnormalities were not found at necropsy of any animal of acute toxicity studies. Clinical findings during 14-day observation period included diarrhoea, few faeces, soiling of the anogenital area, and reductions in weight.

Neurotoxicity studies are summarised in *2.6.7*. The NOAEL for neurotoxicity derived from acute neurotoxicity study (*Anonymous, 2012a*) was set at 1000 mg/kg bw/day based on the decreased locomotor activity (total distance, basic and fine movement) in the top dose group (2000 mg/kg bw/day). No other treatment-related clinical or FOB observations were revealed. At necropsy, all tissues in females were within normal limits; an enlarged testis was observed in one male of 1000 mg/kg bw /day dose group (microscopic examination was not performed). No lesions in neural tissues were found

2.6.2.10.2 Comparison with the CLP criteria regarding STOT SE (specific target organ toxicity-single exposure)

According to CLP criteria (Regulation (EC) No. 1272/2008), an active substance is classified in Category 1 or 2 for specific target organ toxicity – single exposure (STOT SE) based on the results of animal studies if it elicits significant and/or severe toxic effects of relevance to the human health at generally low or moderate exposure concentrations, respectively. The toxic effects relating to STOT SE include changes which have affected the function or morphology of a tissue/organ, or have produced serious changes to the biochemistry or haematology of the organism. The Category 3 for STOT SE includes only narcotic effects and respiratory tract irritation. In studies with daminozide, no above mentioned effects were revealed. Furthermore, in the acute oral and dermal toxicity study high doses of the test substance (5000 mg/kg bw/day), which exceeded CLP guidance dose ranges for STOT SE classification, were used. The acute inhalation study was conducted using the highest attainable dose.

2.6.2.10.3 Conclusion on classification and labelling for STOT SE (specific target organ toxicity-single exposure)

According to CLP criteria (Regulation (EC) No. 1272/2008), the classification is not required.

RAC evaluation of specific target organ toxicity – single exposure (STOT SE)

Summary of the Dossier Submitter's proposal

The DS proposed no classification of daminozide for specific target organ toxicity – single exposure due to the lack of toxic effects meeting the classification criteria.

Comments received during public consultation

One manufacturer and one MSCA supported no classification for this endpoint.

Assessment and comparison with the classification criteria

No substance-related mortalities were reported in acute studies via the oral, dermal, and inhalation route of exposure, and no abnormalities were observed at necropsy in any of these studies. Clinical observations post exposure included diarrhoea, few faeces, soiling of the anogenital area, emaciation and reduced bodyweight.

In addition, two neurotoxicity studies are available with daminozide. In the acute neurotoxicity study (Anonymous, 2012a), Crl:CD(SD) rats (10/dose/sex) were exposed to daminozide technical doses of 0, 500, 1000, 2000 mg/kg bw by a single oral gavage. Decreased locomotor activity (total distance, basic and fine movement) was observed in the top dose group (not statistically significant). At necropsy, an enlarged testis was observed in one male of 1000 mg/kg bw dose group (microscopic examination was not performed).

Based on the above assessment, RAC agrees with DS proposal fthat **no classification for STOT SE** is warranted.

2.6.3 Summary of repeated dose toxicity (short-term and long-term toxicity) [section 10.12 of the CLH report]

2.6.3.1 Specific target organ toxicity-repeated exposure (STOT RE)

Table 35: Summary table of animal studies on repeated dose toxicity (short-term and long-term toxicity) STOT RE (specific target organ toxicity - repeated exposure)

Method, guideline, deviations[1] if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results - NOAEL/LOAEL - target tissue/organ - critical effects at the LOAEL	Reference
90-day oral toxicity study OECD Guideline 408 Deviations: uteri were not weighed at necropsy Baseline values of clinical biochemistry and haematological tests were not included EPA OPPTS 870.3100 Rat (Wistar) Males, females 10 animals/group **Acceptable study**	Test material: daminozide Purity: 100.2% Oral route: by gavage Dose levels: 0, 40, 200, 1000 mg/kg bw/day Vehicle: 0.25% CMC (carboxymethylcellulose) Duration of exposure: 90 days	NOAEL: 1000 mg/kg bw/day (top dose) LOAEL: >1000 mg/kg bw/day No adverse effects	*Anonymous (2005)*
1-year oral toxicity study EPA Pant 158 OECD TG 452 Deviations: the addition of fourth group is recommended	Test material: daminozide Purity: 99% Oral route: in diet Dose levels: 0, 300, 3000,	NOAEL: 80.5 mg/kg bw/day (3000 ppm) LOAEL: 199 mg/kg bw/day (7500 ppm) Renal cell adenoma in one	*Anonymous (1988a)*

if 6 – 10 fold intervals between dosages are used Prothrombin and thromboplastin time were not measured Epididymides and uteri were not weighed at necropsy At the beginning of the study, the body weight variation for each sex of animals should not exceed ± 20% of the mean weight, which was not met Dog (Beagle) Males, females 6 animals/group **Acceptable study**	7500 ppm Duration of exposure: 1 year	female dog Food-like emesis, soft stool (days 14 – 26 occasionaly for most of males)	
28-day dermal toxicity study EPA OPPTS 870.3200 OECD TG 410 Rat (Wistar:Clr) Males, Females 10 animals/group **Acceptable study**	Test material: daminozide Purity: 99% Form: powder Dermal route Dose levels: 0, 125, 500, 2000 mg/kg bw/day Vehicle: deionized water Duration of exposure: 28 days	NOAEL: 2000 mg/kg bw/day (top dose) LOAEL: >2000 mg/kg bw/day No adverse effects	*Anonymous (2012)*

Combined chronic toxicity carcinogenicity study OECD 453, EPA OPP 83-2 Deviations: prothrombin time and activated partial thromboplastin time were not investigated Epididymides, uterus, and thyroid were not weighted at necropsy after the chronic toxicity phase Rat (Fischer 344), males and females 60 animals/group; interim sacrifice: 10♀ and 10 ♂ **Acceptable study**	Test material: daminozide Oral route: in diet Dose levels: 0, 100, 500, 5000, 10000 ppm for 24 months Purity: 99% Form: granules	NOAEL (carcinogenicity): could not be stated, the provisional NOAEL of 100 ppm (equivalent to 5 mg/kg/bw day) was derived Non-neoplastic effects: **bile duct hyperplasia** (in males ↑ by 10% at the top dose; in females ↑ by 27.7%, 21%, 27%, 43% at 100, 500, 5000 and 10000 ppm, respectively comparing to control) Neoplastic effects: increased incidence of pituitary adenomas in females (37.3%, 72%, 84.4%, 76%, 46.6% in control, 100, 500, 5000 and 10000 ppm, respectively; significant increase in the incidence of tumours in low and mid-doses)	*Anonymous (1988b)*
Carcinogenicity study OECD 451 Mouse (CD-1) males and females, 50 animals/group **Acceptable study**	Daminozide Oral route: in diet Dose levels: 0 (controls), 300, 3000, 6000 and 10000 ppm for 24 months Purity: 99% Form: granules	NOAEL (carcinogenicity): could not be stated Non-neoplastic effects: **decreased platelet** (at 3000 – 10000 ppm; 24 months) **and erythrocyte count** (at 10000 ppm; 24 months) in females (*see Table 2.6.3.1.1-8)* **inflammation and brown pigmentation of the liver** in males *(see Table 2.6.3.1.1-7)* Neoplastic effects: increased incidence of pulmonary neoplasms (alveolar/bronchiolar adenomas + carcinomas) in both sexes (in	*Anonymous (1988c)*

		males:↑ by 6%, 16%, 26%, 16%; significant at 5000 ppm; in females: by 18%, 18%, 20%, 20% in 100, 500, 5000, 10000 ppm, respectively; significant at two highest doses)	
Prenatal development toxicity study OECD TG 414 Deviations: At the end of the study only 15 and 8 pregnant females were alive in the 500 and 1000 mg/kg group, respectively. However, each test group should contain approximately 20 pregnant females at necropsy, groups with fewer than 16 animals may be inappropriate. Maternal mortality should not exceed 10 percent, which was not met in the study. Rabbit (New Zealand White) 25 females/group **Acceptable study**	Test material: daminozide Oral route: by gavage Dose levels: 0, 250, 500 and 1000 mg/kg bw/day Vehicle: carboxymethyl cellulose (0.5% w/v) Exposure: days 6 to 28 of presumed gestation Purity: 99.5% Form: powder	NOAEL (maternal toxicity): 250 mg/kg bw/day LOAEL (maternal toxicity): 500 mg/kg bw/day Critical effects *(see Table 2.6.6.2.1-6)*: **mortality** (36% vs. 4% in control; $p < 0.05$) and adverse clinical observations (soft/liquid faeces: 80% vs. 36% in control; $p < 0.05$; **hyperpnoea:** 16% vs. 0%; $p < 0.01$; **hyperactivity:** 12% vs. 0% in control; $p < 0.05$; **convulsions:** 12% vs. 0% in control; non-significant) NOAEL (developmental toxicity): 500 mg/kg bw/day based on the slight reduction in ossification *(see Section 2.6.6.2.1 and Tables 2.6.6.2.1-8 and 2.6.6.2.1-9*) and foetal weight on a litter basis (↓ by 15.3%; $p < 0.05$; *see Table 2.6.6.2.1-7*) LOAEL (developmental): 1000 mg/kg bw/day No teratogenic effects were observed	*Anonymous (2006b)*

Table 36: Summary table of human data on repeated dose toxicity STOT RE (specific target organ toxicity-repeated exposure)

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference	Type of data/report
No data available					

Table 37: Summary table of other studies relevant for repeated dose toxicity STOT RE (specific target organ toxicity-repeated exposure)

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference	Type of data/report
No data available					

2.6.3.1.1 Short summary and overall relevance of the provided information on specific target organ toxicity – repeated exposure (short-term and long-term toxicity)

28-day dermal study in rats (*Anonymous, 2012*): No adverse effects were observed at any dose level. Examined parameters included: clinical observations (expanded with detailed CNS evaluation), mortality and moribundity checks, body weight, food consumption, ophthalmoscopy, gross pathology, organ weights, and microscopic pathology. Based on the lack of adverse findings, the NOAEL was set at 2000 mg/kg bw/day.

90-day oral study in rats (*Anonymous, 2005*): No animal died during the study. Thinning fur, hair loss, sores, and staining were occasionally observed across all groups including controls, and are considered to be of no toxicological relevance. No consistent effects of treatment on bodyweight or food consumption were observed *(see Table 2.6.3.1.1-1 and 2.6.3.1.1-2)*. In Females of the top dose group (1000 mg/kg/day) increased spleen weight adjusted to overall mean necropsy body weight was observed. In the absence of any histopathological findings, this observation is considered not to be of toxicological significance (*see Table 2.6.3.1.1-3)*. Females at the top dose also showed significantly higher blood calcium level (outside the historical control range), which was not, however, considered to be of biological relevance as no signs of hypercalcemia such are nausea, vomiting, constipation or excessive urination were observed *(see Table 2.6.3.1.1-4)*. At urinalysis, both sexes treated with 1000 mg/kg bw/day had the significantly increased specific gravity (outside the historical control range in males); males also showed significantly reduced volumes, slightly darker urine of lower pH with amorphous debris *(see Table 2.6.3.1.1-5)*.. Results of urinalysis were not supported by macroscopic or histopathological changes in kidneys and seem to be caused by the decreased water consumption. Nevertheless, the water consumption of animals was not followed. Additionally, males treated with 200 mg/kg bw/day had a lower concentration of phosphates *(see Table 2.6.3.1.1-4)*.. On the basis that there were no findings of toxicological significance, the NOAEL is considered to be 1000 mg/kg bw/day. Differences in clinical biochemistry parameters (e.g. calcium concentration) or organ weights observed between the top dose and control group were only minor (although statistically significant) and not supported by accompanying macroscopic, histopathological or behavioural findings.

Table 2.6.3.1.1-1: Group mean body weight (g), (*Anonymous, 2005)*

Dose [mg/kg bw/day]	0		40		200		1000	
	♂	♀	♂	♀	♂	♀	♂	♀
Start	165.6	136.3	164.1	133.5	162.9	136.6	165.6	135.4
Week 1	207.5	152.7	201.8	149.3	202.5	152.7	205.7	153.3
Week 3	280.1	178.6	268.3	177.3	266.8	180.9	271.6	183.5
Week 5	327.4	200.5	315.6	195.7	309.7	200.5	315.8	202.3
Week 7	365.4	211.4	345.9	209.8	337.4	214.2	350.7	221.5
Week 9	381.7	217.3	367.0	216.0	356.4	221.3	364.3	226.6
Week 11	400.6	223.2	383.2	223.8	370.1	225.0	378.9	233.0
Week 13	405.1	221.6	389.1	220.7	374.5	224.2	380.1	231.2
(% of control)	(100%)	(100%)	(96.1%)	(99.6%)	(92.4%)	(101.2%)	(93.8%)	(104.3%)

Table 2.6.3.1.1-2: Group mean food consumption over selected periods (g/animal/week), no statistically significant changes observed (ANOVA); (*Anonymous, 2005)*

Dose [mg/kg bw/day]	0		40		200		1000	
	♂	♀	♂	♀	♂	♀	♂	♀
Week 1 – 4	196.5	145.8	210.3	144.6	206.6	151.6	195.5	150.8
Week 5 – 8	207.0	142.3	208.4	144.4	193.1	150.7	194.8	146.0
Week 9 – 13	181.2	133.5	183.2	138.6	179.8	137.7	183.5	148.4
Week 1 – 13	193.8	140.0	199.3	142.2	192.1	146.0	190.7	148.7

Table 2.6.3.1.1-3: Group mean organ weight adjusted to overall mean necropsy body weight, (*Anonymous, 2005)*

Dose [mg/kg bw/day]	0		40		200		1000	
	♂	♀	♂	♀	♂	♀	♂	♀
Adrenals (g)	0.075	0.067	0.073	0.071	0.082	0.067	0.068	0.071
Kidney (g)	1.950	1.266	1.979	1.193	1.890	1.202	1.916	1.322
Spleen (g)	0.705	0.491	0.765	0.501	0.743	0.531	0.716	0.588*
Liver (g)	8.600	5.529	9.061	5.449	8.863	5.449	8.756	5.769
Heart (g)	1.054	0.733	1.053	0.707	1.059	0.702	1.036	0.750
Brain (g)	2.001	1.839	1.962	1.836	2.017	1.825	2.014	1.865
Thyroids (g)	0.016	0.014	0.019	0.014	0.019	0.013	0.018	0.014
Thymus (g)	0.362	0.315	0.350	0.289	0.371	0.308	0.350	0.312
Testes (g)	5.461		5.555		5.542		5.568	
Ovaries (g)		0.084		0.090		0.083		0.103

*p<0.05

Table 2.6.3.1.1-4: Group mean clinical chemistry parameters (*Anonymous, 2005)*

Dose [mg/kg bw/day]	0		40		200		1000	
	♂	♀	♂	♀	♂	♀	♂	♀
AST (IU/L)	68	75	64	70	62	74	63	68
ALT (IU/L)	33	32	35	38	34	39	31	36
ALP (IU/L)	177	88	171	85	167	97	164	84
Na (mmol/l)	140	144	142	143	140	143	140	143
K (mmol/l)	5.8	4.8	5.3	4.8	4.9	4.8	5.1	4.6
Ca (mmol/l)	2.69	2.82	2.67	2.84	2.66	2.83	2.70	2.93**
P (mmol/l)	2.2	1.6	1.9	1.5	1.8**	1.4	2.1	1.7
Glucose (mmol/l)	6.6	6.1	6.0	5.4	6.2	5.4	6.4	4.8**
Urea (mmol/l)	8.7	8.6	7.3	9.1	7.1	8.6	7.1	8.7

Dose [mg/kg bw/day]	**0**		**40**		**200**		**1000**	
	♂	♀	♂	♀	♂	♀	♂	♀
Bilirubin (umol/l)	2.1	1.9	1.8	1.9	2.2	1.9	2.6	2.6
Creatinine (umol/l)	88	79	71	72	69	74	68	73

** p<0.01

Table 2.6.3.1.1-5: Group mean urinalysis parameters (*Anonymous, 2005)*

Dose [mg/kg bw/day]	**0**		**40**		**200**		**1000**	
	♂	♀	♂	♀	♂	♀	♂	♀
Volume (ml)	5.5	2.7	4.7	2.6	4.0	2.9	2.3***	2.4
Specific gravity	1.037	1.038	1.036	1.043	1.041	1.038	1.064***	1.053**
pH	7	6	7	6	6	5	5	5

** p<0.01, *** p<0.001

1-year oral study in dogs (*Anonymous, 1988a)*: One female at the top dose (7500 mg/kg food) died from acute haemorrhagic enteritis. The NOAEL is set at 3000 mg/kg food (equal to 80.5 – 82.8 mg/kg bw/day) based on the occurrence of renal cell adenoma in one female dog, and higher incidence of food-like emesis and soft stool in both sexes of the top dose.

Carcinogenicity studies (*see 2.6.5*): In the rat study (*Anonymous, 1988b*), an increase in the incidence of bile duct hyperplasia was found in the treated females (from the lowest dose) compared to the controls which might be related to the administration of the test article. However, the incidence of bile duct hyperplasia in treated males did not differ from the incidence in controls, which was high (about 70 – 80%; *see Table 2.6.3.1.1-6*). Based on the microscopic evaluation, bile duct hyperplasia was graded as trace or mild in the most of effected animals. According to the literature data, bile duct hyperplasia in rats occurs commonly with age. In the F344 strain, this spontaneous change is often mild, frequently associated with mild fibrosis, or inflammation. And there is neither evidence that it causes significant alteration of hepatic function, nor progresses to cancer (*Greenblatt, 1982; Eustis, 1990*). No other treatment-related signs of hepatotoxicity were evident in either sex.

In the study with mice (*Anonymous, 1988c*), the inflammation as well as brown pigmentation of the liver was more prevalent in the treated than in control males (*see Table 2.6.3.1.1-7*). The inflammation in the liver was predominantly multifocal, mild – moderate, and chronic in nature. Special stains of the brown pigment were not performed, but it appeared to be a mixture of both hemosiderin and bile pigment. The signs of hepatotoxicity were also followed in studies with UDMH (daminozide metabolite, *see bellow and 2.6.8.1*).

The statistically significant decrease in the mean platelet count was observed in females at three highest doses (3000, 6000, 10000 ppm) at the end of the study. Although this parameter is highly variable in rodents, the pattern of

occurrence was indicative of a test article-related effect. Females at the highest group also showed significantly decreased erythrocyte count (*see Table 2.6.3.1.1-8*).

Table 2.6.3.1.1-6: Summary of histopathological findings in the liver (*Anonymous, 1988b*); ( ) = number of examined animals, DOS = died on study, SAC = scheduled sacrifice;

Dose	**0 ppm**		**100 ppm**		**500 ppm**		**5000 ppm**		**10000 ppm**	
	DOS	**SAC**	**DOS**	**SAC**	**DOS**	**SAC**	**DOS**	**SAC**	**DOS**	**SAC**
Males	(25)	(24)	(26)	(23)	(18)	(32)	(24)	(26)	(12)	(37)
Bile duct hyperplasia	17	19	17	16	11	27	18	21	10	31
Overall (%)	73.5		67.3		76		78		83.7	
Females	(15)	(35)	(12)	(37)	(13)	(37)	(11)	(39)	(15)	(35)
Bile duct hyperplasia	1	4	3	13	2	11	6	10	2	12
Overall (%)	5		32.7		26		32		48	

Table 2.6.3.1.1-7: The incidence of inflammation and brown pigmentation of the liver (*Anonymous, 1988c*); ( ) = number of examined animals, DOS = died on study, SAC = scheduled sacrifice;

INCIDENCE OF INFLAMMATION OF THE LIVER		0 ppm		300 ppm		3000 ppm		6000 ppm		10000 ppm	
		DOS	SAC	DOS	SAC	DOS	SAC	DOS	SAC	DOS	SAC
	Males	(29)	(21)	(26)	(24)	(26)	(24)	(33)	(17)	(35)	(15)
		0	3	2	6	1	8	7	5	5	5
	Females	(27)	(23)	(31)	(19)	(25)	(25)	(29)	(21)	(31)	(19)
		2	17	2	10	3	9	2	5	9	10
INCIDENCE OF BROWN PIGMENT IN THE LIVER		0 ppm		300 ppm		3000 ppm		6000 ppm		10000 ppm	
		DOS	SAC	DOS	SAC	DOS	SAC	DOS	SAC	DOS	SAC
	Males	(29)	(21)	(26)	(24)	(26)	(24)	(33)	(17)	(35)	(15)
		1	1	1	3	2	2	8	4	11	3
	Females	(27)	(23)	(31)	(19)	(25)	(25)	(29)	(21)	(31)	(19)
		3	2	3	2	6	5	1	4	5	3

Table 2.6.3.1.1-8: Haematological changes (*Anonymous, 1988c*); ( ) = number of examined animals; * p<0.05, ** p<0.01

	0 ppm			**300 ppm**			**3000 ppm**			**6000 ppm**			**10000 ppm**		
month	12	18	24	12	18	24	12	18	24	12	18	24	12	18	24
Leukocytes, x10^3/cmm															
Males	6.9 (10)	5.2 (10)	5.7 (10)	7.7 (10)	5.7 (10)	**9.7*** (10)	7.9 (10)	7.6 (10)	7.0 (10)	7.3 (10)	7.9 (9)	7.9 (10)	5.3 (10)	6.9 (10)	6.2 (10)
Females	4.4 (10)	5.0 (10)	6.9 (10)	4.4 (10)	5.4 (10)	7.2 (10)	5.5 (10)	5.6 (10)	10.6 (10)	5.1 (10)	4.8 (10)	6.1 (10)	5.0 (10)	5.8 (10)	5.2 (10)
Erythrocytes, x 10^6/cmm															
Males	7.8 (10)	7.6 (10)	7.3 (10)	7.6 (10)	7.4 (10)	7.6 (10)	7.9 (10)	7.6 (10)	7.0 (10)	7.6 (10)	7.1 (9)	7.1 (10)	7.4 (10)	**6.7*** *(10	7.0 (10)
Females	7.49 (10)	7.19 (10)	7.07 (10)	7.71 (10)	6.70 (10)	6.67 (10)	7.52 (10)	7.18 (10)	7.03 (10)	7.48 (10)	6.91 (10)	6.51 (10)	7.60 (10)	6.63 (10)	**5.83**** (10)
Haematocrit, %															
Males	39.4 (10)	39.2 (10)	39.0 (10)	38.4 (10)	37.8 (10)	40.5 (10)	39.5 (10)	38.4 (10)	37.9 (10)	38.8 (10)	**36.2 **(9)	36.9 (10)	37.8 (10)	**34.3 *(10)	37.2 (10)
Females	39.2 (10)	38.2 (10)	38.1 (10)	40.6 (10)	35.0 (10)	37.5 (10)	39.1 (10)	37.5 (10)	38.8 (10)	38.5 (10)	36.3 (10)	36.5 (10)	38.8 (10)	**34.7** *(10)	33.2 (10)
Haemoglobin, g/dl															
Males	14.7 (10)	14.4 (10)	13.7 (10)	14.4 (10)	13.7 (10)	14.4 (10)	14.8 (10)	14.3 (10)	13.4 (10)	14.4 (10)	**13.3 **(9)	13.2 (10)	13.9 (10)	**12.8** *(10)	13.2 (10)
Females	14.6 (10)	14.0 (10)	13.3 (10)	14.9 (10)	12.9 (10)	13.2 (10)	14.5 (10)	13.8 (10)	13.7 (10)	14.4 (10)	13.5 (10)	12.8 (10)	14.4 (10)	12.9 (10)	11.8 (10)
Platelets, x10^3/cmm															
Males	903 (9)	1223 (10)	1169 (10)	994 (8)	1152 (9)	1084 (9)	886 (10)	1000 (10)	1131 (10)	791 (10)	1096 (9)	856 (9)	973 (10)	1003 (10)	919 (10)
Females	850 (9)	778 (10)	877 (10)	878 (8)	773 (10)	756 (10)	807 (10)	794 (10)	**594*** *(10)	**732*** (10)	694 (10)	**576*** *(10)	774 (10)	646 (10)	**415**** (10)

Teratogenicity study in rabbits (*Anonymous, 2006b*): Increased mortality, hyperactivity and convulsions were observed in females at the dose of 500 and 1000 mg/kg bw/day (*see 2.6.6.2*). Therefore, the NOAEL was set at 250 mg/kg bw/day.

Neurotoxicity studies (*see 2.6.7*): No adverse effects relating to STOT RE were observed.

Studies on UDMH (daminozide metabolite; *see Section 2.6.8.1*): In 90-day subchronic mouse study *(Anonymous, 1987b)*, liver hypertrophy, karyomegaly, and accentuation of lobulation occurred in all treated male groups (already at the lowest dose of 10 ppm equal to 2 mg/kg bw/day), *(see Table 2.6.8.1-1)*. In 2-year rat carcinogenicity study *(Anonymous, 1989a)*, hepatocellular neoplasms observed in females at all dose levels (0.1 – 8 mg/kg bw/day) were associated with chronic inflammation of the liver. In 2-year mouse carcinogenicity study (*Anonymous, 1989b*), conducted with lower levels (ranging from 0.2 – 2.7 mg/kg bw/day equal to 1 – 20 ppm), the incidence of brown pigment in the liver was increased in the treated mice from the dose of 5 ppm. Special stains were not performed to determine the type of the pigment. The pigmentation seemed to consist predominantly of lipofuscin, which is associated with aging in many organs. The bile pigment, which is an indicative of hepatotoxicity, was probably present as well. In the second 2-year mouse carcinogenicity study *(Anonymous, 1990)*, conducted with higher dose levels (40 and 80 ppm equal to 7.3 and 21.8 mg/kg bw/day, respectively), the significant increase in the incidence of neoplastic lesions in the liver (haemangiomas/ haemagiosarcomas) observed at all treated groups was associated with signs of hepatotoxicity (accentuated liver lobulation, liver cell hypertrophy and necrosis, presence of chronic inflammation and brown pigment, elevated levels of alanine aminotransferase and sorbitol dehydrogenase; *see Table 2.6.8.1-6 and 2.6.8.1-7*). However, the excessive mortality (*see Table 2.6.8.1-8)* in this study indicates that the dosing was probably set over the maximum tolerated dose (MTD).

2.6.3.1.2 Comparison with the CLP criteria regarding STOT RE (specific target organ toxicity-repeated exposure)

According to CLP criteria (Regulation (EC) No. 1272/2008), an active substance is classified in Category 1 or 2 for the specific target organ toxicity – repeated exposure (STOT RE) based on the results of animal studies if it elicits significant and/or severe toxic effects of relevance to the human health at generally low or moderate exposure concentrations, respectively. The toxic effects relating to STOT RE include changes which have affected the function or morphology of a tissue/organ (e.g. necrosis, fibrosis, granuloma formation, steatosis), or have produced serious changes in the biochemistry or haematology. In 28 and 90-day studies with daminozide, no adverse effects relating to STOT RE were observed. In carcinogenicity studies, bile duct hyperplasia in female rats, inflammation as well as brown pigmentation of the liver in male mice, and the decrease in haematological parameters (platelet and erythrocyte count in female mice) were shown. Nevertheless, these changes were not considered to be severe enough for STOT RE classification. In addition, adverse effects observed in mice from the dose of 300 ppm equal to 35 mg/kg bw/day were not noted within the critical range of doses/concentrations for classification in Category 2 (i.e. $1.6 < C \leq 16.6$ mg/kg bw/day, if Haber´s rule is considered for 90-day exposure in mice; for 2-year exposure, the levels would be even lower). The treatment-related effects noted in rabbits of teratogenicity study were considered to be appropriate for the setting of NOAEL/LOAEL, but not for STOT RE classification because 1000 mg/kg bw/day causing excessive mortality is according to OECD TG 414 the limit dose. Moreover, hyperactivity and convulsion were not observed in any study with daminozide (including neurotoxicity studies). In studies with daminozide metabolite (UDMH), more significant

signs of hepatotoxicity (necrosis, elevated levels of alanine aminotransferase and sorbitol dehydrogenase) were revealed at doses exceeding maximum tolerated dose.

2.6.3.1.3 Conclusion on classification and labelling for STOT RE (specific target organ toxicity-repeated exposure)

No classification according to CLP criteria (Regulation (EC) No. 1272/2008) is proposed.

RAC evaluation of specific target organ toxicity – repeated exposure (STOT RE)

Summary of the Dossier Submitter's proposal

The DS proposed no classification for specific target organ toxicity – repeated exposure (STOT RE). In repeated dose toxicity studies with rats via dermal and oral application route, no adverse effects fulfilling the criteria for STOT RE were identified. Bile duct hyperplasia in female rats, as well as inflammation/brown pigmentation of the liver in male mice and changes in some haematological parameters (reduced platelet and erythrocyte count in female mice) were reported in carcinogenicity assays with daminozide. Severity of these effects was considered insufficient for STOT RE classification. Increased mortality, hyperactivity and convulsions were observed at 500 and 1000 mg/kg bw/day in teratogenicity study with rabbits, however these effects were not seen in any other study (including neurotoxicity studies), and 1000 mg/kg bw/day causing excessive mortality is considered the limit dose according to OECD TG 414. *In vivo* animal studies indicated that at least 1% of the substance is metabolized to UDMH, therefore its toxicity profile was considered in this assessment. Chronic studies with UDMH in mice reported significant liver toxicity (accentuated liver lobulation, liver cell hypertrophy and necrosis, presence of chronic inflammation and brown pigment, elevated levels of alanine aminotransferase and sorbitol dehydrogenase) at doses of 7.3 and 21.8 mg/kg bw/day. Excessive mortality rates (up to 98%) indicate that the dosing in this study exceeded the maximum tolerated dose (MTD). Considering all the available data the DS proposed no classification for STOT RE.

Comments received during public consultation

One manufacturer supported no classification for STOT RE.

Assessment and comparison with the classification criteria

In a 28-day toxicity study according to OECD TG 410, Wistar rats (10/sex/dose) were exposed to daminozide doses of 0, 125, 500 and 2000 mg/kg bw/day via dermal application (Anonymous, 2012). No adverse effects were reported at any dose level.
In a 90-day toxicity study according to OECD TG 408, Wistar rats (10/sex/dose) were treated with daminozide at dose levels of 0, 40, 200, 1000 mg/kg bw/day via oral gavage (Anonymous, 2005). No deaths or any significant effects on bodyweight and food consumption were reported during the study. The adjusted (to bodyweight) spleen weight was increased in females at 1000 mg/kg bw/day without corresponding histopathological findings. Blood calcium

level was significantly higher (and slightly outside Historical Control Data (HCD)) in females at the top dose, however without typical signs of hypercalcemia such are nausea, vomiting, constipation or excessive urination. Urine in both sexes had significantly increased specific gravity, and significantly reduced volumes, darker colour, and amorphous debris were reported in males. Since there were no macroscopic or histopathological changes in the kidneys, effects on urine were linked to decreased water consumption. It is noted, however, that water intake during the study was not monitored. A lower concentration of blood phosphates was observed in males treated with 200 mg/kg bw/day (Table below). Overall, the observed small (although statistically significant) differences in clinical biochemistry or organ weights were not accompanied by macroscopic, histopathological or behavioural findings, and were not considered toxicologically relevant.

***Table**: Selected clinical chemistry and urine analysis parameters (Anonymous, 2005)*

Dose (mg/kg bw/day)	**0**		**40**		**200**		**1000**	
	♂	♀	♂	♀	♂	♀	♂	♀
Clinical chemistry								
Ca (mmol/L)	2.69	2.82	2.67	2.84	2.66	2.83	2.70	2.93*
P (mmol/L)	2.2	1.6	1.9	1.5	1.8*	1.4	2.1	1.7
Glucose (mmol/L)	6.6	6.1	6.0	5.4	6.2	5.4	6.4	4.8*
Urinalysis parameters								
Volume (mL)	5.5	2.7	4.7	2.6	4.0	2.9	2.3**	2.4
Specific gravity	1.037	1.038	1.036	1.043	1.041	1.038	1.064**	1.053*
pH	7	6	7	6	6	5	5	5

* p<0.01, ** p<0.001

In a 1-year oral toxicity study according to OECD TG 452, beagle dogs (6/sex/dose) were treated with daminozide at doses of 0, 300, 3000, 7500 ppm (corresponding to 0, 8.5, 80.5, 199 mg/kg bw/day) via food administration (Anonymous, 1988a). One female at the top dose (199 mg/kg bw/day) died from acute haemorrhagic enteritis. Renal cell adenoma in one female dog and higher incidence of food-like emesis and soft stool were reported in both sexes of the top dose. While renal cell adenoma are rare tumours in dog, the single occurrence of this finding was considered spontaneous since no accompanying macroscopic, histopathological or biochemical changes in the kidneys were observed.

In a combined chronic toxicity and carcinogenicity study according to OECD TG 453, Fischer rats (60/sex/dose, 10 ♀/♂ at scheduled interim sacrifice) received daminozide via food at dose levels of 0, 100, 500, 5000, 10000 ppm (corresponding to 0, 5, 25, 250, 500 mg/kg bw/day) for 24 months (Anonymous, 1988b). The incidence of bile duct hyperplasia was increased in all treated females compared to the controls, while the rates in males were similar among all groups, and notably higher than in females (no statistical significance reported). Severity was graded as trace or mild. No other treatment-related signs of hepatotoxicity were evident in either sex. In addition, higher incidences of ovarian atrophy and ovarian cysts were reported in in all treated females without clear dose relation. Information on severity is not available.

***Table**: Incidence of bile duct hyperplasia observed in a combined chronic toxicity and carcinogenicity study with daminozide (Anonymous, 1988b)*

Dose ppm (mg/kg bw/day)	**0 ppm (0)**		**100 ppm (5)**		**500 ppm (25)**		**5000 ppm (250)**		**100 (500)**	
Time of examination	DOS	SAC	DOS	SAC	DOS	SAC	DOS	SAC	DOS	SAC
Males examined	25	24	26	23	18	32	24	26	12	37
Bile duct hyperplasia	17	19	17	16	11	27	18	21	10	31
Overall	73.5%		67.3%		76%		78%		83.7%	
Females examined	15	35	12	37	13	37	11	39	15	35
Bile duct hyperplasia	1	4	3	13	2	11	6	10	2	12
Overall	10%		32.7%		26%		32%		28%	

DOS=died on study, SAC=scheduled sacrifice

In a carcinogenicity study according to OECD TG 451, CD-1 mice (50/sex/dose) were exposed to daminozide via the food at doses of 0, 300, 3000, 6000 and 10000 ppm (corresponding to 0, 45, 450, 900, and 1500 mg/kg bw/day) for 24 months (Anonymous, 1988c). Liver inflammation (multifocal, mild to moderate) and brown pigmentation (presumably a mixture of hemosiderin and bile pigment) were reported in all treated males. In females, the mean platelet count at ≥3000 ppm and the erythrocyte count at the top dose were statistically significant decreased. The adversity of these changes is not considered to be severe enough to trigger a STOT RE classification.

In a prenatal development toxicity study according to OECD TG 414, increased mortality, hyperactivity and convulsions were observed in rabbits at doses of 500 and 1000 mg/kg bw/day (Anonymous, 2006b). Mortality was preceded by adverse clinical observations and/or reductions in body weight gain and feed consumption.

***Table**: Mortality and clinical observations in a prenatal development toxicity study with rabbits (Anonymous, 2006b)*

Dose (mg/kg bw/day)	**0**	**250**	**500**	**1000**
Animals per group	24	24	24	24
Mortality	1	1	9*	14**
Found dead	0	0	7 (29%)**	8 (33%)**
Moribund sacrificed	1 (4%)	1 (4%)	2 (8%)	6 (25%)
Clinical observations				
Hyperactivity	0	0	3*	5**
Convulsion	0	0	3	3
Ptosis	0	0	0	4**
Aborted and sacrificed	0	0	0	2 (8%)

*p<0.05, **p<0.01;

No signs of neurotoxicity and systemic toxicity relating to STOT RE were observed in a subchronic neurotoxicity toxicity study according to OECD TG 424 where Crl:CD(SD) rats received daminozide via oral gavage at doses of 0, 100, 300, 1000 mg/kg bw/day for 90 days (Anonymous, 2012b).

According to CLP criteria, substances are classified for specific target organ toxicity following repeated exposure based on findings of significant and/or severe toxicity at low to moderate doses. Considering the weight of evidence from all of the above studies, RAC supports the view of DS that no classification for STOT RE is warranted. The increased mortality and convulsions

observed in the teratogenicity study with rabbits at ≥500 mg/kg bw/day were not reported in other oral studies with similar dosing (Anonymous, 2005; Anonymous, 2012b). Thus, their toxicological significance remains unclear for the assessment of this endpoint. In addition, the lowest dose with significant mortality reported (500 mg/kg bw/day) falls outside of the extrapolated guidance value for classification in STOT RE 2 based on 22 days exposure duration (ca. 400 mg/kg bw/day, Haber's rule). The most significant finding in terms of STOT RE classification are the increased incidence of bile duct hyperplasia observed in the females from the chronic study with rats at ≥100 ppm (5 mg/kg bw/day). This dose level falls within the adjusted range of guidance values of 1.25<C≤12.5 mg/kg bw/day for classification in Category 2 on a base of a 24-month study (Haber's rule). It is noted, however, that bile duct hyperplasia occurs spontaneously in the F344 strain, frequently associated with mild fibrosis or inflammation but without adverse change in hepatic function (e.g. cholestasis) or progresses to cancer. The occasional occurrence of bile duct hyperplasia in the absence of any differences in incidence or severity among study groups likely represents a background lesion (NTP neoplastic lesions atlas). In the present study, the unusually high incidence of bile duct hyperplasia in the males of the control group (73.5% vs. 10% for females) complicates the interpretation of the data. Commonly associated lesions such as inflammation, fibrosis, or bile duct dilation were not reported. Considering that severity was graded as trace or mild for most of the affected animals, and the lack of a clear dose response of the data, **RAC concludes that classification for STOT RE is not warranted.**

2.6.4 Summary of genotoxicity / germ cell mutagenicity [equivalent to section 10.8 of the CLH report template]

Table 38: Summary table of genotoxicity/germ cell mutagenicity tests *in vitro*

Method, guideline, deviations[1] if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations /Results	Reference
Bacterial reverse mutation assay with *E. coli* WP2uvr A (Ames test) OECD Guideline 471 Deviations: 2-aminoanthracene was used as the only indicator of S9 mix efficacy Number of cells/culture was not reported **Acceptable study**	Daminozide Purity: 100.2% Form: powder	Test concentrations: Experiment 1 (plate incorporation): 0, 1.6, 8, 40, 200, 1000, 5000 µg/plate in the presence and absence of S9 mix, in triplicates Experiment 2 (pre-incubation): 0, 156.25, 312.5, 625, 1250, 2500, 5000 µg/plate in the presence and absence of S9 mix, in triplicates Positive control: 4-nitroquinoline-N-oxide; 2-aminoanthracene Negative control: vehicle (purified water)	The test was negative in the presence as well as absence of S9 mix No cytotoxicity observed Positive and vehicle control: valid	*Williams (2006)* Report No. 2242/50
Bacterial reverse mutation assay with *Salmonella typhimurium* TA 1535, TA 1537, TA 1538, TA 98 and TA 100 (Ames test) OECD Guideline 471 Deviations: the recommended maximum test	Daminozide Purity: 99.8% Form: solid	Test concentrations: Range finding test: 10, 33, 67, 100, 333, 667, 1000, 3333, 6667, 10000 µg/plate Mutation tests: 667, 1000, 3333, 6667, 10000 µg/plate Positive control: 2-nitrofluorene; sodium azide; ICR-191, 2-aminoanthracene Negative control: vehicle	The test was negative in the presence as well as absence of S9 mix No cytotoxicity observed (tested up to limit concentrations) Positive and vehicle control: valid	*San (1991)* Report No. A.7.6.18

Method, guideline, deviations[1] if any	**Test substance**	**Relevant information about the study including rationale for dose selection (as applicable)**	**Observations /Results**	**Reference**
concentration for non-cytotoxic substances is 5mg/plate and not 10mg/plate Bacterial strain for detection of crosslinking agents was not used, however Ames test with Escherichia is available (*Williams (2006)* 2-aminoanthracene was used as the only indicator of S9 mix efficacy The plates should be incubated at 37 ºC (37 ±2 ºC in the study) **Acceptable study**		(DMSO)		
Bacterial reverse mutation assay with *Salmonella typhimurium* TA 1535, TA 1537, TA 98 and TA 100 Deviations: only 4 bacterial strains were used; the	Daminozide Purity: 99% Form: powder	Test concentrations: Range finding test: 5, 50, 500 and 5000 µg/plate Mutation tests: 50, 150, 500, 1500 and 5000 µg/plate Positive control: 2-nitrofluorene; 9-aminoacridine; sodium	The test was negative in the presence as well as absence of S9 mix No cytotoxicity observed (tested up to limit concentrations) Positive and vehicle	*Richold (1984)* Report No. FNA 4/84222

Method, guideline, deviations[1] if any	**Test substance**	**Relevant information about the study including rationale for dose selection (as applicable)**	**Observations /Results**	**Reference**
strain for detection of crosslinking mutagens was not involved 2-aminoanthracene was used as the only indicator of S9 mix efficacy **Supplementary study**		azide; 2-aminoanthracene Negative control: vehicle (water)	control: valid	
TK+/- mouse lymphoma cell mutation assay OECD Guideline 476 **Acceptable study**	Daminozide Purity: 99%	Test concentrations: Preliminary experiment: 1.95, 3.9, 7.81, 15.63, 31.25, 62.5, 125, 250, 500,1000, 2000, 3000, 4000 µg/ml Main experiment: 0, 1500, 2000, 2333.3, 2666.7, and 3000 µg/mL chosen on the basis of cytotoxicity in the preliminary experiment Positive control: DMBA, Ethylmethanesulphonate Negative control: water	The test was negative in the presence as well as absence of S9 mix Positive and negative control: valid	*Bootman (1982b)* Report No. A.7.6.5
In vitro mammalian chromosome aberration test with CHO cells OECD Guideline 473 Deviations: a short term treatment in the absence of S9 mix was not	Daminozide Purity: 99.8% Form: powder	Test concentrations: Preliminary cytotoxicity assay +/- S9 mix: 0, 0.2, 0.6, 2, 6, 20, 60, 200, 600, and 2000 µg/mL Chromosomal aberration assay +/- S9 mix: 0, 250, 500, 1000 and 2000 µg/mL Positive control:	The test was negative in the presence as well as absence of S9 mix Positive and vehicle control: valid	*Putman (1991)* Report No. A.7.6.19

Method, guideline, deviations[1] if any	**Test substance**	**Relevant information about the study including rationale for dose selection (as applicable)**	**Observations /Results**	**Reference**
performed The incubation with test substance lasted 2 hours instead of 3-6 hours Less than 300 metaphases were scored **Acceptable study**		triethylenemelamine; cyclophosphamide Negative control: vehicle (DMSO)		
DNA damage and/or repair with *E. Coli* strains: WP 2, WP 67, CM 871 OECD TG not available **Supplementary study**	Daminozide Purity: 99% Form: crystalline	Test concentrations: 250, 1000, 2500, and 10000 µg/mL in the presence and absence of S9 mix for 24 hours Positive control: mitomycin C; 2-aminoanthracene Negative control: deionised watetr	The test was negative in the presence as well as absence of S9 mix	*Bootman (1982a)* Report No. A.7.6.6
Mitotic aneuploidy (non-disjunction) assay in yeast OECD TG not available **Supplementary study**	Daminozide Purity: 99% Form: powder	Test concentrations: 1, 2, 5, 10, 20, 50, 100, 200, 500, 1000, and 2000 µg/ml in the presence and absence of S9 mix for 12 hours Positive control: 12-0-Tetradeconoylphorbol-13-acetate (TPA) and deoxycholate	The test was negative in the presence as well as absence of S9 mix	*Bootman (1983)* Report No. A.7.6.7

Table 39: Summary table of genotoxicity/mutagenicity tests in mammalian somatic or germ cells *in vivo*

Method, guideline, deviations[1] if any	**Test substance**	**Relevant information about the study (as applicable)**	**Observations/Results**	**Reference**
Combined *in vivo* micronucleus and chromosome aberration test OECD TG 474 and 475 Deviations: 2000 immature erythrocytes (instead of 4000)/animal were evaluated for incidence of micronuclei 100 (instead of 200) metaphases/animal were analysed for chromosomal aberrations Intraperitoneal administration is not recommended **Acceptable study**	Daminozide Purity: 99.39% Form: crystalline powder	Mouse (ICR) Intraperitoneal route 7 groups, 5 ♂ and 5 ♀/group Test concentrations: ♂: 500, 1000, 2000 mg/kg; ♀: 375, 750, 1500 mg/kg chosen on the basis of the pilot study Animals euthanized 22-24 or 46-48 hours after treatment Negative control: water Positive control: cyclophosphamide monohydrate	No statistically significant increase in the number of aberrant or micronucleated cells was observed relative to respective controls	*Anonymous (2003)*
DNA binding study No OECD TG available **Supplementary study**	Radiolabelled daminozide Purity: not stated	Rat (Sprague-Dawley), 2 ♂ Oral: 37 mg/kg (4.7 mCi/kg) by gavage Animals euthanized 24 hours after treatment DNA from liver analysed	daminozide contributed to DNA radioactivity by 6% (via DNA methylation); CBI=0.5 (biosynthetic incorporation of radiolabelled nucleotide precursors into DNA was taking into account);	*Anonymous(1986)*

Method, guideline, deviations[1] if any	Test substance	Relevant information about the study (as applicable)	Observations/Results	Reference
			Daminozide did not damage DNA via covalent binding to a relevant extent	
Dominant lethal assay The assay was performed prior to adoption of OECD Guideline 478 **Supplementary study**	Daminozide Purity: not stated	Mice, 20 ♂ 10, 300, and 1000 mg/kg/food (equivalent to 0, 1.5, 45, and 150 mg/kg bw/day) for 5 days	No treatment-related effects on mating, pregnancy rate, embryonic deaths and implantation loss	*Anonymous* (*1973)*

Table 40: Summary table of human data relevant for genotoxicity / germ cell mutagenicity

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference	Type of data/report
No data available					

2.6.4.1 Short summary and overall relevance of the provided information on genotoxicity / germ cell mutagenicity

Bacterial reverse mutation assay (Ames test): Three tests, performed in compliance with OECD 471, are available. The studies *San (1991)* and *Richold (1984)* used *Salmonella typhimurium* strains with GC base pair at the primary revision site i.e. TA 1535, TA 1537, TA 98, and TA 100 (TA 1538 only in *San, 1991*). *Salmonella typhimurium* strain with AT base at the primary revision site, e.g. TA 102 for detection of oxidizing and cross-linking agents was not involved, however the standalone test with *Escherichia coli* strain WP2uvr A was conducted (*Williams, 2006).*

In studies with *Salmonella typhimurium* strains the concentrations of the test substance were chosen on the basis of the preliminary range finding tests. No cytotoxicity was observed up to limit concentrations of 5 mg/plate and 10 mg/plate, respectively. Positive and negative controls were valid. The increase in revertant colony number was not observed in any strain at the concentration of 50 – 10000 µg/plate in the presence as well as absence of metabolic activation (Aroclor 1254-induced rat liver post-mitochondrial fraction S9)), (*see Tables 2.6.4.1-1 – 2.6.4.1-4).*

Table 2.6.4.1-1: Mean number ± SD of revertant colonies obtained in the initial mutation assay *(San, 1991)*

	Mean revertant colony counts									
	Without metabolic activation					**With metabolic activation**				
Dose level (µg/plate)	TA 98	TA 100	TA 1535	TA 1537	TA 1538	TA 98	TA 100	TA 1535	TA 1537	TA 1538
0	23±5	128±4	11±1	4±2	8±2	33±3	153±10	14±2	8±2	13±2
667	21±4	138±7	15±2	7±3	5±1	27±3	141±5	11±3	6±2	9±1
1000	26±4	160±12	13±5	5±1	6±1	27±8	146±18	14±3	6±1	8±2
3333	25±6	149±18	12±4	7±0	9±2	19±4	152±19	14±4	6±0	10±3
6667	30±12	141±7	8±0	9±3	5±1	32±4	139±18	16±3	6±5	11±2
10000	23±3	146±10	12±6	6±4	10±5	26±7	143±10	16±3	5±3	11±3
Positive controls										
2NF	212±50	-	-	-	321±14	-	-	-	-	-
NaN_3	-	474±27	254±19	-	-	-	-	-	-	-
ICR-191	-	-	-	115±10	-	-	-	-	-	-
2AA	-	-	-	-	-	100±32	1901±115	45±9	32±4	255±19

2NF: 2-nitrofluorene; NaN_3: sodium azide; 2AA: 2-aminoanthracene; ICR-191: CAS 1707-45-0

Table 2.6.4.1-2: Mean number ± SD of revertant colonies obtained in the confirmatory mutation assay *(San, 1991)*

	Mean revertant colony counts									
	Without metabolic activation					**With metabolic activation**				
Dose level (µg/plate)	TA98	TA 100	TA 1535	TA 1537	TA 1538	TA98	TA 100	TA 1535	TA 1537	TA 1538
0	15±3	127±5	8±3	7±3	35±6	15±2	146±5	13±5	6±3	35±4
667	13±2	125±21	7±2	5±1	37±10	14±2	145±9	15±4	6±4	42±2
1000	12±2	131±4	8±4	7±1	35±11	17±1	112±17	14±1	7±1	32±10
3333	15±5	117±11	9±3	7±2	36±5	18±4	138±12	13±1	7±4	38±4
6667	9±3	118±10	10±4	4±2	32±3	20±2	131±4	9±1	8±2	34±8
10000	11±0	125±6	6±5	7±3	33±3	18±2	129±7	14±3	6±5	40±3
Positive controls										
2NF	161±36	-	-	-	313±23	-	-	-	-	-
NaN_3	-	415±27	363±20	-	-	-	-	-	-	-
ICR-191	-	-	-	49±4	-	-	-	-	-	-
2AA	-	-	-	-	-	1389±168	1341±18	114±15	172±6	1305±63

2NF: 2-nitrofluorene; NaN_3: sodium azide; 2AA: 2-aminoanthracene; ICR-191: CAS 1707-45-0

Table 2.6.4.1-3: Mean number ± SD of revertant colonies obtained in the initial mutation assay *(Richold, 1984)*

	Mean ± SD revertant colony counts							
	Without metabolic activation				**With metabolic activation**			
Dose level (µg/plate)	Strain TA1535	Strain TA1537	Strain TA98	Strain TA100	Strain TA1535	Strain TA1537	Strain TA98	Strain TA100
5000	10±4.6	4±1.5	43±2.6	65±11.6	4±1.5	5±-[a]	30±2.6	76±18.4
1500	8±1.5	9±2.9	40±7.9	60±5.5	7±4.2	15±4.0	25±5.8	73±9.5
500	9±2.0	12±4.6	43±2.6	68±12	12±4.7	27±1.2	39±3.1	90±3.1
150	8±3.5	10±4.0	29±2.1	65±16.5	6±3.2	20±1.5	34±3.6	97±8.5
50	11±1.0	16±4.0	32±5.7	78±10.0	9±3.1	14±2.6	27±1.2	95±2.3
0	12±5.5	10±2.3	37±4.9	71±7.9	10±1.0	20±4.5	27±3.5	85±13.1
Positive controls								
2AA	-	-	-	-	115±17.0	158±11.3	1195±125.4	702±43.7
2NF	-	-	475±25.7	-	-	-	-	-
9AAC	-	125±34.4	-	-	-	-	-	-
NaN_3	889±11.4	-	-	795±107.4	-	-	-	-

2NF: 2-nitrofluorene; NaN_3: sodium azide; 2AA: 2-aminoanthracene; 9AAC: 9-aminoacridine; [a]: contaminated

Table 2.6.4.1-4: Mean number ± SD of revertant colonies obtained in the confirmatory mutation assay *(Richold, 1984)*

	Mean ± SD revertant colony counts							
	Without metabolic activation				**With metabolic activation**			
Dose level (µg/plate)	Strain TA1535	Strain TA1537	Strain TA98	Strain TA100	Strain TA1535	Strain TA1537	Strain TA98	Strain TA100
5000	7±2.3	13±3.6	27±6.7	95±9.5	8±2.5	19±9.5	27±4.4	90±8.1
1500	8±1.0	16±1.7	22±3.6	85±11.0	10±0.6	18±2.3	20±1.5	83±28.7
500	7±2.1	14±4.0	27±5.7	82±12.1	11±2.6	20±1.0	18±2.1	79±18.2
150	10±6.2	13±2.5	30±2.5	70±4.5	8±1.5	19±1.5	22±5.5	85±16.6
50	5±2.0	16±3.5	27±3.5	81±16.6	4±1.2	22±1.5	23±2.9	88±14.5
0	10±4.0	20±0.6	26±8.5	80±3.1	14±2.5	18±3.2	21±2.5	75±13.1
Positive controls								
2AA	-	-	-	-	98±11.8	124±11.1	564±22.2	870±24.8
2NF	-	-	378±84.5	-	-	-	-	-
9AAC	-	104±28.4	-	-	-	-	-	-
NaN_3	441±38.4	-	-	411±29.1	-	-	-	-

2NF: 2-nitrofluorene; NaN_3: sodium azide; 2AA: 2-aminoanthracene; 9AAC: 9-aminoacridine;

The study with *Escherichia coli* (*Williams, 2006)* was carried out in 2 experiments. In the first one, no evidence of toxicity was observed up to 5000 µg/plate. Narrowed concentration intervals in the second one experiment were used

in order to more closely investigate concentration ranges approaching the limit concentration (156.25 to 5000 µg/plate), and therefore considered most likely to provide evidence of any mutagenic activity. Following these treatments, no evidence of toxicity was observed. The mean numbers of revertant colonies on negative control plates fell within acceptable ranges, and were significantly elevated by positive control treatments. No statistically significant increases in revertant colonies were observed after treatment with daminozide in the absence or presence of metabolic activation *see Table 2.6.4.1-4).*

Table 2.6.4.1-4: Mean number ± SD of revertant colonies *(Williams, 2006)*

Treatment	**Dose (µg/ plate)**	**Revertant colonies/plate (mean ± SD)**			
		Experiment 1		**Experiment 2**	
		-S9	**+S9**	**-S9**	**+S9**
Daminozide	0	7 ± 3	11 ± 4	13 ± 2	20 ± 4
	1.6	8 ± 2	7 ± 3		
	8	10 ± 4	13 ± 2		
	40	5 ± 1	6 ± 1		
	156.25			18 ± 6	24 ± 5
	200	6 ± 4	11 ± 3		
	312.5			16 ± 3	19 ± 6
	625			14 ± 12	20 ± 6
	1000	8 ± 4	15 ± 3		
	1250			16 ± 6	18 ± 4
	2500			11 ± 1	15 ± 7
	5000	8 ± 3	6 ± 2	15 ± 9	19 ± 6
Positive controls	NQO	1188 ± 74		986 ± 264	
	AAN		254 ±17		99 ± 17

AAN = 2-aminoanthracene, NQO = 4-nitroquinoline-1-oxide;

In vitro mammalian chromosome aberration test (*Putman, 1991*): The test was performed on CHO cells in the presence and absence of metabolic activation (S9 mix) according to OECD TG 473. A short treatment with the test substance in the absence of S9 mix was not included. The test material caused no increase in chromosome aberrations with or without metabolic activation. All negative control cultures gave values of chromosomal aberrations within the expected range. Positive controls (triethylenemelamine in the absence and cyclophosphamide in the presence of metabolic activation) induced marked increases in the incidence of structurally aberrant cells. Based on these findings, the test substance did not exert any potential to induce chromosomal aberrations in CHO cells *(see Table 2.6.4.1-5).*

Table 2.6.4.1-5: Summary of results *(Putman, 1991)*

Group	Dose (µg/mL)	Mitotic index (%)	Cells scored	Aberrations per cell (mean)	Cells with aberrations (%)
Without metabolic activation					
Control	Untreated	6.4	100	0.020±0.141	2
	DMSO	6.5	100	0.040±0.197	4
Daminozide	250	7.1	100	0.010±0.100	1
	500	6.5	100	0.010±0.100	1
	1000	6.9	100	0.000±0.000	0
	2000	6.8	100	0.010±0.100	1
TEM	0.5	2.4	100	0.250±1.114	12**
With metabolic activation					
Control	Untreated	10.6	100	0.000±0.000	0
	DMSO	9.6	100	0.010±0.100	1
Daminozide	250	10.6	100	0.030±0.171	3
	500	10.4	100	0.000±0.000	0
	1000	10.2	100	0.030±0.171	3
	2000	11.2	100	0.000±0.000	0
CP	50	3.1	100	0.220± 1.040	13**

TEM: Triethylenemelamine, CP: Cyclophosphamide; **: p≤0.01 at Fisher's exact test;

In vitro mammalian cell gene mutation test (*Bootman, 1982b*): The aim of the study was to evaluate the potential of the test substance to induce mutations in L5178Y mouse lymphoma cells, which are heterozygous at the thymidine kinase gene locus (TK+/-). The study was performed according to guideline OECD 476. In the presence as well as absence of S9 mix, a sharp reduction in the cell growth was observed between test material concentrations of 2000 and 3000 µg/mL, from 100% growth at 2000 µg/m1 to 12.5 or 13.0% growth at 3000 µg/ml (preliminary experiment). The solvent control mutation frequencies were within the range of historical controls. After the treatment with positive control, the mutation frequencies were enhanced. Daminozide did not induce significantly increased mutation frequencies *(see Table 2.6.4.1-6).*

Table 2.6.4.1-6: Mutation frequency in L5178Y mouse lymphoma cells *(Bootman, 1982b)*

Treatment [ppm]	Mutation frequency per 10^5 surviving cells	Induced mutation frequency	Total growth [%]
Metabolic activation	**-S9/S9+**	**-S9/S9+**	**-S9/S9+**
Control (DH_2O)	5.8/5.2	0.0/0.0	100.0/100.0
1500	7.4/5.2	1.6/0.0	75.0/70.6
2000	7.2/5.9	1.4/0.7	75.4/40.1
2333.3	5.9/7.4	0.1/2.2	44.7/34.2
2666.7	8.5/6.5	2.7/1.3	31.1/47.4
3000	8.0/8.3	2.2/3.1	16.7/34.0
EMS (300)	44.1/-	38.3/-	16.4/-
DMBA (5)	7.4/51.3	1.6/46.1	33.3/13.4

EMS=Ethylmethanesulphonate, DMBA=7,12 dimethylbenzanthracene

Combined *in vivo* micronucleus and chromosome aberration assay (*Anonymous, 2003)*: The assay was performed according to OECD TG 474 and 475, and represents the key *in vivo* genotoxicity study. The pilot phase was designed to assess the toxicity of the test article and set dose levels for the definitive study. The definitive study was designed to evaluate the potential of the test article to increase the incidence of micronucleated polychromatic erythrocytes (MPCEs) and chromosome aberrations in bone marrow of male and female ICR mice. Since differences in the toxicity between the sexes were observed, the high dose for the definitive study was set at 2000 mg/kg for male and 1500 mg/kg for female mice. No mortality was observed during the definite study. Clinical signs of systemic toxicity (piloerection and lethargy) were noted at 1000 mg/kg and above. The mitotic index was reduced in male treated groups at the 22 – 24 hours sampling time (up to 16%) while no appreciable reductions were observed in the treated female groups at the same time as well as in both sexes at the 46 – 48 hours sampling time. No statistically significant increase in the number of aberrant cells was observed in the treated groups relative to the negative controls regardless of dose level or bone marrow sampling time ($p>0.05$ Fisher's exact test). No significant increase in the number of MPCEs in the treated groups comparing to the negative control was observed either at 22 – 24 or 46 – 48 hours after dose administration ($p>0.05$, Kastenbaum-Bowman Tables). A single intraperitoneal administration of the test material at doses up to 2000 mg/kg (males) or 1500 mg/kg (females) induced neither significant increase in the incidence of micronucleated polychromatic erythrocytes*(see Table 2.6.4.1-9)* nor numerical and structural chromosome aberrations in the bone marrow cells *(see Table 2.6.4.1-7 and 2.6.4.1-8).*

Table 2.6.4.1-7: Summary of *in vivo* chromosome aberration results (22 – 24 h post dose), *(Anonymous, 2003)*

SDC: Cells having at least 10 aberrations of any type, including pulverized chromosomes or cells;

[a]: number of cells in mitosis per 1000 cells observed, expressed as a percentage (MI)

Group	Dose (mg/kg)	Sex	Cells scored	Mean mitotic index (%)[a]	Cells with aberrations		Struct. Aberr. (%)	Number of aberrations			SDC	Aberrations per cell (mean)
					Num.	Struct.		Gap	Break	Exch.		
Control (water)	-	M	500	11.3	0	2	0.4	0	2	0	0	0.004±0.005
		F	500	10.1	0	1	0.2	0	1	0	0	0.002±0.004
Daminozide	500	M	500	10.2	0	0	0.0	0	0	0	0	0.000±0.000
	375	F	500	10.0	0	0	0.0	0	0	0	0	0.000±0.000
	1000	M	500	10.4	0	0	0.0	0	0	0	0	0.000±0.000
	750	F	500	10.3	0	2	0.4	0	2	0	0	0.004±0.005
	2000	M	500	9.5	0	2	0.4	0	2	0	0	0.004±0.005
	1500	F	500	10.6	0	0	0.0	0	0	0	0	0.000±0.000
CP	50	M	500	6.1	0	61*	12	0	24	0	530	1.108±0.188
	50	F	500	6.4	0	61*	12	0	42	1	470	1.026±0.228

Table 2.6.4.1-8: **Summary of *in vivo* chromosome aberration results (46 – 48 h post dose*);*** SDC = Cells having at least 10 aberrations of any type, including pulverized chromosomes or cells; CP = cyclophosphamide; ***(Anonymous, 2003)***

Group	Dose (mg/kg)	Sex	Cells scored	Mean mitotic index (%)	Cells with aberrations		Struct. Aberr. (%)	Number of aberrations			SDC	Aberrations per cell (mean)
					Num.	Struct.		Gap	Break	Exch.		
Control (water)	-	M	500	10.9	0	0	0.0	0	0	0	0	0.000±0.000
		F	500	10.3	0	0	0.0	0	0	0	0	0.000±0.000
Daminozide	2000	M	500	10.0	0	3	0.6	0	3	0	0	0.006±0.009
	1500	F	500	11.1	0	0	0.0	0	0	0	0	0.0000±0.000

Table 2.6.4.1-9: Summary of *in vivo* micronucleus test *(Anonymous, 2003)*

Treatment	Dose (mg/kg)	Sex	Time (h)	PCE/total erythrocytes (mean ± SD)	Micronucleated PCE [a] (mean ± SD)
Control (water)	0	M	22-24	0.509 ± 0.05	0.5 ± 0.00
	0	F		0.423 ± 0.06	0.5 ± 0.00
Daminozide	500	M		0.451 ± 0.03	0.3 ± 0.27
	375	F		0.446 ± 0.01	0.4 ± 0.22
	1000	M		0.434 ± 0.03	0.6 ± 0.22
	750	F		0.373 ± 0.04	0.8 ± 0.27
	2000	M		0.302 ± 0.04	1.2 ± 0.57
	1500	F		0.335 ± 0.03	0.9 ± 0.42
CP	50	M		0.318 ± 0.02	22.2 ± 3.82*
	50	F		0.339 ± 0.03	23.0 ± 4.77*
Control (water)	0	M	46-48	0.510 ± 0.02	0.5 ± 0.00
	0	F		0.478 ± 0.01	0.4 ± 0.22
Daminozide	2000	M		0.176 ± 0.03	1.2 ± 0.57
	1500	F		0.233 ± 0.10	0.5 ± 0.35

*: statistically significant, $p<0.05$ (Kastenbaum-Bowman Tables); [a]: micronucleated PCEs per 1000 PCEs

PCEs: polychromatic erythrocytes; CP: cyclophosphamide

Investigation of the potential for covalent binding of daminozide to rat liver DNA *(Anonymous, 1986)*: The study was performed neither in compliance with any guideline nor GLP. The covalent binding of the test material to DNA of target cells was studied *in vivo* by analysing the DNA isolated from liver of two male rats to which radio labelled test material had been administrated. About 6% of the DNA radioactivity co-chromatographed with 7-methylguanine. The results indicated that the radioactivity associated with the DNA, as determined 24 hours after oral administration of the radiolabelled test material, was mostly due to biosynthetic incorporation of radiolabelled nucleotide precursors into DNA and that methylation of liver DNA by the test material contributed little to the overall DNA radioactivity. The extent of this DNA damage, expressed in units of the Covalent Binding Index, CBI (CBI = µmol chemical bound per mol nucleotide/mmol chemical applied per kg body weight), was in the order of 0.5 for the test material. Compounds with CBI: (i) > 1000 are regarded as potent carcinogens; (ii) of the order of 100 as moderately strong genotoxic carcinogens; (iii) < 10 weakly genotoxic carcinogens; If the CBI < 1, it is unlikely that the substance will induce tumours via DNA binding. Therefore, the test material is considered to be negative in the present study.

Dominant lethal assay *(Anonymous, 1973)*: The study was not performed according to any standardised guideline as it was accomplished prior to adoption of OECD guideline 478. The study was not performed under GLP conditions. The test material was administered to the test animals via the diet route; four groups of male mice (n = 20) were treated with 0, 10, 300, and 1000 mg/kg food (equivalent to 0, 1.5, 45, and 150 mg/kg bw/day) for 5 consecutive days. The mating period lasted one week and there were 4 matings in total. Male animals were observed for signs of toxicity, body weight and food consumption. Females were examined to determine total implantations, viable embryos, and early and late deaths. No treatment-related effects on mating performance, pregnancy rate, embryonic deaths and implantation loss were observed. Thus, the test substance did not induce dominant lethal effects in germ cells of male mice under conditions of the present study (the tested doses were rather low).

In summary, daminozide did not induce gene mutations either in bacterial reverse mutation assay (Ames test) with strains of *Escherichia coli* and *Salmonella typhimurium* or mammalian cell gene mutation test with TK+/- mouse lymphoma cells. The test substance was also negative in chromosome aberration study with Chinese hamster ovary cells. The *in vivo* chromosome aberration study combined with micronucleus test using bone marrow cells revealed that daminozide did not increase either the incidence of chromosomal aberrations or micronucleated polychromatic erythrocytes in mice. Based on the negative results of *in vitro* as well as *in vivo* studies, daminozide is considered to exert no genotoxic properties. This conclusion is also supported by the findings of the supplementary material showing that daminozide does not have the potential of covalent binding to DNA. However, it has to be taken into account that the purity of daminozide was not stated in this study.

2.6.4.2 Comparison with the CLP criteria regarding genotoxicity / germ cell mutagenicity

According to CPL criteria (Regulation (EC) No. 1272/2008), the classification in the last category for genotoxicity (Category 2) is based on positive evidence obtained from *in vivo* somatic cell mutagenicity tests in mammals or other *in vivo* somatic cell genotoxicity tests which are supported by positive results from *in vitro* mutagenicity assays. All genotoxicity tests performed with daminozide were negative.

2.6.4.3 Conclusion on classification and labelling for genotoxicity / germ cell mutagenicity

As all genotoxicity studies with daminozide showed a negative result, the classification according CLP criteria (Regulation (EC) No. 1272/2008) is not required.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

The DS proposed no classification for germ cell mutagenicity based on clearly negative results from several *in vitro* and *in vivo* genotoxicity tests.

In vitro, daminozide did not induce gene mutations in three bacterial reverse mutation assays (Ames test) with strains of *Escherichia coli* and *Salmonella typhimurium*. A gene mutation test with TK+/- mouse lymphoma cells was also negative. Further, a chromosome aberration study with Chinese hamster ovary cells was negative.

In vivo, daminozide did not increase either the incidence of chromosomal aberrations or micronucleated polychromatic erythrocytes in a chromosome aberration study combined with micronucleus test using bone marrow cells in mice. Further *in vivo* studies demonstrated that daminozide does not have the potential of covalent binding to DNA. The DS concluded that the classification criteria for germ cell mutagenicity are not fulfilled.

Comments received during public consultation

Three MSCAs and one manufacturer commented on this endpoint during public consultation. Two MSCAs and the manufacturer supported no classification for germ cell mutagenicity. One MSCA pointed out that *in vitro* mutagenicity studies (Ames test) using liver S9 mix may not have been suitable to predict *in vivo* genotoxicity potential of daminozide since *in vitro* metabolism in hepatocytes was very limited. Another MSCA noted further that incubation time of daminozide in the cell culture might have been too short and thus not optimal for generation of potentially genotoxic metabolite UDMH.

Assessment and comparison with the classification criteria

Three bacterial reverse mutation assays (Ames test) performed in compliance with OECD TG 471, are available. The studies by San (1991) and by Richold (1984) used *Salmonella typhimurium* strains TA 1535, TA 1537, TA 98, and TA 100 (TA 1538 only in San, 1991), while a standalone test with *Escherichia coli* strain WP2uvr was performed by Williams (2006). Positive and negative controls were included in the study, and no cytotoxicity was observed up to the limit concentrations of 5 mg/plate (Richold 1984, Williams 2006) and 10 mg/plate (San, 1991), respectively. No increase in the number of revertant colonies was reported in any strain at concentrations of 50 – 10000 µg/plate in the presence as well as absence of metabolic activation (Tables 2.6.4.1-1 – 2.6.4.1-4 in CLH report).

In vitro mammalian chromosome aberration test (Putman, 1991) in CHO cells was performed in the presence and absence of metabolic activation (S9 mix) according to OECD TG 473.

Deviations from the guideline included the lack of a short-term treatment in absence of S9 mix, 2 hours incubation with test substance instead of 3-6 hours, and scoring of less than 300 metaphases. Cyclophosphamide and triethylenemelamine were used as positive controls for treatments with and without S9 mix, respectively. No increase in chromosomal aberrations was observed in tests with or without metabolic activation.

In vitro mammalian cell gene mutation test according to OECD TG 476 was performed in L5178Y mouse lymphoma cells heterozygous at the thymidine kinase gene locus (TK+/-) (Bootman, 1982b). Ethylmethanesulphonate and 7,12-dimethylbenzanthracene were used as positive controls, and a sharp reduction in the cell growth was observed between test concentrations of 2000 and 3000 µg/mL. Daminozide did not induce significantly increased mutation frequencies in L5178Y mouse lymphoma cells (Table 2.6.4.1-6 in CLH report).

A combined *in vivo* micronucleus and chromosome aberration assay was performed in mice according to OECD TG 474 and 475 (Anonymous, 2003). ICR mice (5/sex/dose) were treated with daminozide at dose levels of 500, 1000 and 2000 mg/kg bw for males, and 375, 750 and 1500 mg/kg bw for females via single intraperitoneal injection. Cyclophosphamide monohydrate at 50 mg/kg was used as a positive control. Reported deviations from the guideline include analysis of 2000 (instead of 4000) immature erythrocytes/animal and of 100 (instead of 200) metaphases/animal for chromosomal aberrations. No mortality was reported during the study, and clinical signs of systemic toxicity (piloerection and lethargy) was observed after dosing above 1000 mg/kg bw. The mean mitotic index was reduced in the top dose males at 22-24 hours (9.5 vs. 11.3 in control), while no changes were observed in females at the same time, and in both sexes at 46-48 hours. There was no statistically significant increase in the numerical and structural chromosome aberrations and in the number of micronucleated PCEs at any dose and at any sampling time (Tables 2.6.4.1-7, 8 and 9 in CLH report).

The potential for covalent binding of daminozide to the rat liver DNA was investigated in a non-guideline (no GLP) study where two male SD rats received a single dose of 37 mg/kg bw (4.7 mCi/kg bw) radiolabelled substance by oral gavage (Anonymous, 1986). Animals were euthanized 24 hours after treatment and the DNA from the liver was analysed.

The results indicated that methylation of liver DNA by the test material contributed little (6%) to the overall DNA radioactivity. The calculated Covalent Binding Index (CBI) as a measure of DNA damage (CBI = µmol chemical bound per mol nucleotide/mmol chemical applied per kg body weight) was in the order of 0.5. Potent, moderate, and weak carcinogens have CBI values of >1000, ca. 100, and <10, respectively. For CBI<1, it is considered unlikely that the substance will induce tumours via DNA binding, and the test is regarded negative.

In a non-guideline (no GLP) dominant lethal assay (Anonymous, 1973), male mice (5/dose) were treated with daminozide (purity not stated) at concentrations of 0, 10, 300, and 1000 mg/kg in food (equivalent to 0, 1.5, 45, and 150 mg/kg bw/day) for 5 consecutive days. The mating period lasted one week, and there were 4 matings in total. No treatment-related effects on mating performance, pregnancy rate, embryonic deaths and implantation loss were observed. It was concluded that under conditions of the study the test substance did not induce dominant lethal effects in germ cells of male mice. It is noted, however, that the tested doses were rather low.

Based on the above studies, RAC concludes that there was no increase in mutant frequencies in bacterial cells, no increase in chromosomal aberrations or in mutant frequencies in mammalian cells. It is noted that incubation time of daminozide in the cell cultures might have

been too short and thus not optimal for generation of potentially genotoxic metabolite UDMH. Test results *in vivo* in mice showed neither micronucleus induction nor chromosomal damage. Thus, RAC supports the DS opinion that daminozide does not meet the criteria and that **no classification for germ cell mutagenicity is warranted.**

2.6.5 Summary of long-term toxicity and carcinogenicity [equivalent to section 10.9 of the CLH report template]

Table 41: Summary table of animal studies on long-term toxicity and carcinogenicity

Method, guideline, deviations[1] if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results** **- NOAEL/LOAEL** **- target tissue/organ** **- critical effects at the LOAEL**	**Reference**
Combined chronic toxicity carcinogenicity study OECD 453, EPA OPP 83-2 Deviations: prothrombin time and activated partial thromboplastin time were not investigated Epididymides, uterus, and thyroid were not weighted at necropsy after the chronic toxicity phase Rat (Fischer 344), males and females 60 animals/group; interim sacrifice: 10♀ and 10 ♂ **Acceptable study**	Daminozide Oral route: in diet Dose levels: 0 (controls), 100, 500, 5000, 10000 ppm for 24 months Purity: 99% Form: granules	NOAEL (carcinogenicity): could not be stated, the provisional NOAEL of 100 ppm (equivalent to 5 mg/kg/ bw/ day) was derived Non-neoplastic effects: bile duct hyperplasia (in males ↑ by 10% at the top dose; in females ↑ by 27.7%, 21%, 27%, 43% at 100, 500, 5000 and 10000 ppm, respectively comparing to control; see Table 2.6.5.1-3) **Neoplastic effects: increased incidence of pituitary adenomas in females** (37.3%, 72%, 84.4%, 76%, 46.6% in control, 100, 500, 5000 and 10000 ppm, respectively; significant increase in the incidence of tumours in low and mid-doses; see Table 2.6.5.1-4)	*Anonymous (1988b)*
Carcinogenicity study OECD 451 Mouse (CD-1) males and females, 50	Daminozide Oral route: in diet	NOAEL (carcinogenicity): could not be stated Non-neoplastic effects: decreased platelet (at 3000 – 10000 ppm; 24 months) and erythrocyte count (at 10000 ppm; 24 months)	*Anonymous (1988c)*

Method, guideline, deviations[1] if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results** **- NOAEL/LOAEL** **- target tissue/organ** **- critical effects at the LOAEL**	**Reference**
animals/group **Acceptable study**	Dose levels: 0 (controls), 300, 3000, 6000 and 10000 ppm for 24 months Purity: 99% Form: granules	in females (*see Table 2.6.3.1.1-5)*, inflammation and brown pigmentation of the liver in males *(see Table 2.6.3.1.1-4)* **Neoplastic effects: increased incidence of pulmonary neoplasms (alveolar/bronchiolar adenomas + carcinomas) in both sexes** (in males:↑ by 6%, 16%, 26%, 16%; significant at 5000 ppm; in females: by 18%, 18%, 20%, 20% in 100, 500, 5000, 10000 ppm, respectively; significant at two highest doses; see Table 2.6.5.1-9)	

Table 42: Summary table of human data on long-term toxicity and carcinogenicity

Type of data/report	**Test substance**	**Relevant information about the study (as applicable)**	**Observations**	**Reference**	**Type of data/report**
No data available					

Table 43: Summary table of other studies relevant for long-term toxicity and carcinogenicity

Type of data/report	**Test substance**	**Relevant information about the study (as applicable)**	**Observations**	**Reference**	**Type of data/report**
No data available					

2.6.5.1 Short summary and overall relevance of the provided information on long-term toxicity and carcinogenicity

2-year oral carcinogenicity studies were performed in rats and mice according to OECD TG 453 and 451, respectively.

2-year carcinogenicity study in rats (*Anonymous, 1988b)*: No treatment-related clinical signs, effect on ophthalmoscopy, haematology, biochemistry, and urinalysis or any evidence of neurotoxicity were observed. The number of survivors at week 104 was in excess of 50%, thus fully adequate for assessment of carcinogenic potential. The mortality was higher for males than for females and occurred mainly during the last nine months of the study (weeks 66-105) for the control as well as treated groups *(see Table 2.6.5.1-1)*. A small number of significant changes in absolute and/or relative organ

weights were seen, which were not considered to be treatment-related *(see Table 2.6.5.1-2).* An increase in hepatic bile hyperplasia was found in the treated female rats comparing to the controls and may have been related to the administration of the test article *(see Table 2.6.5.1-3).* No other signs of hepatic toxicity were evident in either sex. A slight increase in the incidence of ovarian atrophy was present in the treated females compared to the controls and may have also been related to the administration of the test article *(see Table 2.6.5.1-3).* A large number of spontaneous neoplastic lesions were evident in the study *(see Table 2.6.5.1-4).* However, higher incidence of pituitary adenomas in females, observed from the lowest test group comparing to the concurrent control, was regarded as the treatment-related effect. Therefore, the NOAEL for carcinogenicity could not be set and only the provisional NOAEL of 100 ppm (equivalent to 5 mg/kg bw/day) was derived.

Although F344 rats are considered to be susceptible to developing pituitary adenomas, there are still strains e.g. Sprague-Dawley with higher spontaneous incidence of this neoplasia (42 vs. 78% in female F344 and Sprague-Dawley rats, respectively, *Hayes, 2014*; supported by other studies: *Sandusky, 1988*; reviewed in *Lines, 2016*). Furthermore, in F344 rats, type of tumours with much higher spontaneous incidence occur (e.g. 84% occurrence of Leydig cell neoplasia in males, *Hayes, 2014*). According to data from the toxicology textbook (*Hayes, 2014*), the spontaneous pituitary adenoma occurs in 42% of female F344 rats, which is supported by other studies (36%: *Sandusky, 1988*; 44%: *Haseman, 1984; see Table 2.6.5.1-5 and 2.6.5.1-6*). In the study with daminozide, the pituitary adenoma incidence of female controls correlated with literature data, whereas it was increased at each test dose (statistically significantly at mid-doses; *see Table 2.6.5.1-4).* The relevance and reliability of literature data is evaluated in *Table 2.6.5.1-5*. The data from the toxicology textbook (*Hayes, 2014*) are of lower relevance as the studies on which they are based were performed more than 5 year later than the study with daminozide. Nevertheless, the incidence of pituitary adenomas stated in the toxicology textbook is comparable with incidences published in other papers (studies performed in time period as the study with daminozide, i.e. within ±5 years). *Table 2.6.5.1-6* shows variability in results published by *Haseman (1984)*, which were obtained fom different laboratories. It can be seen that the maximum upper observed level in females, i.e. 70% in laboratory "C" is rare (the mean incidence of pituitary adenomas in females was 44% ± 11.4%). Thus, not only the range, but also the frequency of the incidence of observed adverse effects is a significant variable that must be taken into account when it comes to data from public literature or historical controls.

One could object that the increase in the pituitary adenoma incidence was not observed in males. However, females are known to be more prone to this type of neoplasia than males. In the published literature, approximately 25% incidence of pituitary adenomas in males is stated (*Hayes, 2014).* Despite this fact, in the study with daminozide the concurrent controls in males and females were comparable (42 vs. 37%), which might skew the results in males. Nevertheless, in the study with UDMH (the major metabolite of daminozide, *Anonymous, 1989a; see 2.6.8.1*), the incidence of pituitary adenomas and hepatocellular carcinomas was also increased only in rat females. Thus, the oncogenic potential of UDMH was not exerted in rat males as well.

Although the dose-response relationship was not proved and the statistically significant difference from the control was shown only at mid-doses (where lower number of animals were examined), the incidence of pituitary adenomas in females at the top dose was still higher when compared to the concurrent control as well as the spontaneous incidence stated in the literature (36%: *Sandusky, 1988*; 42%: *Hayes, 2014;* 44%: *Haseman, 1984*).

Table 2.6.5.1-1: Survival data: number of survived rats (% survival); % survival was counted excluding the 10 animals/sex/group sacrificed at 12 months of study (*Anonymous, 1988b)*

	0 ppm		**100 ppm**		**500 ppm**		**5000 ppm**		**10000 ppm**	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
total number	60	60	60	60	60	60	60	60	60	60
week 52	59	60	59	59	60	60	60	60	59	60
rats after 1st kill	49	50	49	49	50	50	50	50	49	50
week 80	48	46	47	45	50	48	47	48	48	47
week 92	40	43	39	42	44	44	39	45	46	42
week 104	26/50 (52 %)	36/50 (72 %)	26/50 (52 %)	37/50 (74 %)	33/50 (66 %)	39/50 (78 %)	27/50 (54 %)	40/50 (80 %)	39/50 (78%)	38/50 (76%)
week 105	24/50 (48%)	35/50 (70%)	23/50 (46%)	37/50 (74%)	32/50 (64%)	37/50 (74%)	26/50 (52%)	39/50 (78%)	37/50 (74%)	35/50 (70%)

Table 2.6.5.1-2: Absolute and relative organ weights; significantly different from controls * p≤0.05

organ	**week**	**0 ppm**		**100 ppm**		**500 ppm**		**5000 ppm**		**10000 ppm**	
		♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Testis weight absolute (g)	52	3.35		3.51 *		3.44		3.42		3.35	
	104	5.90		5.76		4.80		6.53		5.85	
Kidney weight absolute (g)	52	3.27	1.92	3.15	1.90	3.21	1.86	3.19	1.98	3.16	1.90
	104	3.77	2.34	3.55	2.36	3.66	2.31	3.69	2.22*	3.81	2.26
Kidney/brain Weight %x10^{-2}	52	1.73	1.12	1.64	1.09	1.67	1.08	1.67	1.16	1.69	1.12
	104	1.96	1.33	1.81 *	1.33	1.96	1.33	1.89	1.25*	1.95	1.29
Heart/body weight %x10	52	2.80	3.44	2.75	3.33	2.81	3.37	2.78	3.41	2.75	3.54
	104	3.37	3.45	3.42	3.48	3.39	3.47	3.15	3.59	3.35	3.72*

Table 2.6.5.1-3: Summary of histopathological findings in the liver and ovaries; ( ) = number of examined animals, DOS = died on study, SAC = scheduled sacrifice

	0 ppm		**100 ppm**		**500 ppm**		**5000 ppm**		**10000 ppm**	
	DOS	**SAC**	**DOS**	**SAC**	**DOS**	**SAC**	**DOS**	**SAC**	**DOS**	**SAC**
Males	(25)	(24)	(26)	(23)	(18)	(32)	(24)	(26)	(12)	(37)
Bile duct hyperplasia	17	19	17	16	11	27	18	21	10	31
Females	(15)	(35)	(12)	(37)	(13)	(37)	(11)	(39)	(15)	(35)
Bile duct hyperplasia	1	4	3	13	2	11	6	10	2	12
Ovarian atrophy	2	0	4	1	6	3	2	3	7	3
Ovarian cysts	2	0	0	5	2	4	2	5	2	6

Table 2.6.5.1-4: Tumour analysis; *p<0.05, **p<0.01, ***p<0.001; terminal rates = observed tumour incidence at terminal kill (including animals dying or sacrificed in extremis during week(s) of terminal kill); overall rates = number of tumour bearing animals/number of animals examined at site;

Tumour analysis - males (ppm)					
	0	**100**	**500**	**5000**	**10 000**
ADRENAL - Pheochromocytoma, benign					
overall rates	8/60 (13.3%)	1/27 (3.7%)	2/19 (10.5%)	3/28 (10.7%)	8/60 (13.3%)
terminal rates	6/25 (24.0%)	1/3 (33.3%)	0/2 (0.0%)	0/4 (0.0%)	4/37 (10.8%)
ADRENAL - Pheochromocytoma, malignant					
overall rates	1/60 (1.7%)	2/27 (7.4%)	3/19 (15.8%)*	5/28 (17.9%)*	4/60 (6.7%)
terminal rates	0/25 (0.0%)	0/3 (0.0%)	2/2 (100%)	4/4 (100%)	4/37 (10.8%)
HAEMOLYMPHORETICULAR SYSTEM – Mononuclear cell leukaemia					
overall rates	19/60 (31.7%)	18/60 (30.0%)	10/60 (16.7%)*	15/60 (25.0%)	16/60 (26.7%)
terminal rates	9/25 (36.0%)	7/26 (26.9%)	6/33 (18.2%)	4/26 (15.4%)	11/37 (29.7%)
MAMMARY GLAND - Fibroadenoma					
overall rates	1/60 (1.7%)	0/60 (0.0%)	2/60 (3.3%)	0/60 (0.0%)	3/60 (5.0%)
terminal rates	1/25 (4.0%)	0/26 (0.0%)	1/33 (3.0%)	0/26 (0.0%)	3/37 (8.1%)
PANCREAS – Islet cell adenoma					
overall rates	2/60 (3.3%)	1/27 (3.7%)	1/18 (5.6%)	1/23 (4.3%)	6/60 (10.0%)
terminal rates	1/25 (4.0%)	1/3 (33.3%)	0/1 (0.0%)	0/0	5/37 (13.5%)
PITUITARY - Adenoma					
overall rates	25/60 (41.7%)	12/31 (38.7%)	17/27 (63.0%)	12/27 (44.4%)	27/59 (45.8%)

terminal rates	11/25 (44.0%)	4/7 (57.1%)	9/10 (90.0%)	3/3 (100%)	19/36 (52.8%)
TESTIS – Interstitial cell tumour, benign					
overall rates	14/60 (23.3%)	8/50 (16.0%)	12/50 (24.0%)	9/50 (18.0%)	3/60 (5.0%)**
terminal rates	1/25 (4.0%)	1/26 (3.8%)	7/32 (21.9%)	2/26 (7.7%)	1/37 (2.7%)
TESTIS – Interstitial cell tumour, malignant					
overall rates	32/60 (53.3%)	38/50 (76.0%)*	33/50 (66.0%)	34/50 (68.0%)	42/60 (70.0%)*
terminal rates	24/25 (96.0%)	25/26 (92.6%)	24/32 (75.0%)	24/26 (92.3%)	36/37 (97.3%)
THYROID – Parafolicular cell adenoma					
overall rates	6/60 (10.0%)	1/27 (3.7%)	1/19 (5.3%)	0/25 (0.0%)	10/60 (16.7%)
terminal rates	5/25 (20.0%)	0/3 (0.0%)	0/2 (0.0%)	0/2 (0.0%)	8/37 (21.6%)
Tumour analysis - females (ppm)					
	0	**100**	**500**	**5000**	**10 000**
ADRENAL – Pheochromocytoma, benign					
overall rates	1/60 (1.7%)	0/13 (0.0%)	0/14 (0.0%)	0/11 (0.0%)	3/59 (5.1%)
terminal rates	0/36 (0.0%)	0/0	0/1 (0.0%)	0/0	2/36 (5.6%)
ADRENAL – Pheochromocytoma, malignant					
overall rates	2/60 (3.3%)	1/13 (7.7%)	0/14 (0.0%)	1/11 (9.1%)	2/59 (3.4%)
terminal rates	1/36 (2.8%)	0/0	0/1 (0.0%)	0/0	2/36 (5.6%)
HEMOLYMPHORETICULAR SYSTEM – Mononuclear cell leukemia					
overall rates	10/60 (16.7%)	7/60 (11.7%)	11/60 (18.3%)	10/60 (16.7%)	11/60 (18.3%)
terminal rates	4/36 (11.1%)	2/37 (5.4%)	6/37 (16.2%)	3/39 (7.7%)	5/36 (13.9%)
MAMMARY GLAND - Fibroadenoma					
overall rates	3/60 (5.0%)	3/60 (5.0%)	0/60 (0.0%)	2/60 (3.3%)	6/60 (10.0%)
terminal rates	3/36 (8.3%)	2/37 (5.4%)	0/37 (0.0%)	2/39 (5.1%)	4/36 (11.1%)
PITUITARY - Adenoma					
overall rates	22/59 (37.3%)	18/25** (72.0%)	27/32*** (84.4%)	19/25*** (76.0%)	27/58 (46.6%)
terminal rates	16/36 (44.4%)	12/12 (100%)	18/18 (100%)	13/13 (100%)	20/36 (55.6%)
THYROID – Parafollicular cell adenoma					
overall rates	1/60 (1.7%)	1/13 (7.7%)	0/14 (0.0%)	1/12 (8.3%)	4/60 (6.7%)
terminal rates	1/36 (2.8%)	0/0	0/1 (0.0%)	0/1 (0.0%)	3/36 (8.3%)
THYROID – Parafollicular cell carcinoma					
overall rates	2/60 (3.3%)	0/13 (0.0%)	0/14 (0.0%)	1/12 (8.3%)	2/60 (3.3%)
terminal rates	1/36 (2.8%)	0/0	0/1 (0.0%)	1/1 (100.0%)	1/36 (2.8%)
UTERUS - Polyp					
overall rates	9/60 (15.0%)	5/21 (23.8%)	8/23 (34.8%)*	8/23 (34.8%)*	8/60 (13.3%)
terminal rates	7/36 (19.4%)	4/8 (50.0%)	7/10 (70.0%)	8/12 (66.7%)	7/36 (19.4%)

Table 2.6.5.1-5: Literature data on the spontaneous incidence of pituitary adenomas in Fischer 344 rat;

Rat strain	Type of Study	Rate ♂/♀	Range ♂/♀	SD ♂/♀	Years	Relevance/ reliability	Reference
Fischer 344	2-year studies	25%/42%	Data not available		Finished 1997	**Lower**: studies performed more than 5 years later than the study with daminozide, i.e. 1985 – 1987)	*Hayes et al., 2014 (Textbook of toxicology)*
Fischer 344 100 ♂ 100 ♀	2-year study	26%/36%	Data not available		Finished 1988	**Lower:** the same rat strain used; performed in time period as the study with daminozide (within ±5 years); however, **the range was not published**	*Sandusky et al., 1988*
Fisher 344 2158 ♂ 2262 ♀	2-year studies	21.7%/44%	Data not available	11.7%/11.4%	Finished 1983	**High:** the same rat strain used; performed in time period as the study with daminozide (within ±5 years); **complete information available**	*Haseman et al., 1984* ***see Table 2.6.5.1-6 for further information***

Table 2.6.5.1-6: Inter-laboratory variability in literature data on the spontaneous incidence of pituitary adenomas in Fischer 344 rat *(Haseman et al., 1984)*; statistically significant differences were not observed

Laboratory	No of studies /No of males	♂ F344 rats Rate	♂ F344 rats Range	No of studies /No of females	♀ F344 rats Rate	♀ F344 rats Range
A	9/439	17%	5 – 29%	9/439	37%	18 – 50%
B	5/249	18%	6 – 28%	5/249	45%	42 – 52%
C	14/699	24%	7 – 52%	15/747	49%	30 – 70%
D	6/340	18%	8 – 41%	6/337	45%	30 – 64%
E	7/344	30%	19 – 44%	7/350	42%	26 – 67%

2-year carcinogenicity study in mice (Anonymous, 1988c): The survival was non-significantly decreased in both sexes (at the highest dose in females and two highest doses in males; *see Table 2.6.5.1-7*). There were no effects of treatment on body weight and food consumption. In females the statistically significant decrease in the mean platelet count was observed at three highest doses (3000, 6000, 10000 ppm) at the end of the study. Although this parameter is highly variable in rodents, the pattern of occurrence was indicative of a test article-related effect. Females at the highest group also showed significantly decreased erythrocyte count (*see Table 2.6.3.1.1-5; Section STOT RE*). Inflammation as well as brown pigmentation in the liver was more prevalent in the treated than in control males and may be related to the administration of the test article (*see Table 2.6.3.1.1-4; Section STOT RE*). The incidence of macroscopic masses/nodules in the lungs was increased in the treated groups comparing to controls *(see Table 2.6.5.1-8).* A variety of neoplastic lesions were seen in both sexes across dose levels. However, the incidence of alveolar/bronchiolar adenomas as well as alveolar/bronchiolar adenomas combined with carcinomas was increased in each treated group in both sexes when compared to the concurrent control. This effect

was considered to be the treatment-related. Therefore, the NOAEL for carcinogenicity could not be derived from this study *(see Table 2.6.5.1-9)*.

The incidence of adenomas in the male concurrent control is within the range of historical controls stated in the study report (18.2 – 44% and 8.7 – 22% in males and females, respectively; *see Table 2.6.5.1-10*). Despite the fact that females are known to be less sensitive to pulmonary tumours than males, the incidence of adenomas in the female control group is the same as in the male one (40%). This value is too high, out of the range of historical controls, does not correlate with the literature data (2 – 42% and 2 – 27% in males and females, respectively; *Giknis, 2005, Hayes, 2014;* the relevance and reliability of literature data on the spontaneous incidence of alveolar/bronchiolar adenomas/carcinomas is evaluated in *Table 2.6.5.1-11* and *Table 2.6.5.1-12*), and may skew the results. Nevertheless, the adenoma incidence is increased above the concurrent as well as historical control in each treated group in both sexes, which is also evident (to a greater extent) after combination of adenoma with carcinoma. Fisher exact test revealed that the increase in the incidence of adenomas and adenomas combined with carcinomas in males at the dose of 6000 ppm as well as adenomas combined with carcinomas in females at two highest doses (6000 and 10000 ppm) is statistically significant compared to the control. Although alveolar/bronchiolar adenoma belongs to the common neoplasms in CD1 male mice *(Chandra, 1992)*, CD1 mice are considered to represent less susceptible strain *(Nikitin, 2004)*. In the highly susceptible mouse strains such as A/J, the onset of pulmonary tumours occurs in 3 – 4 months, followed by 100% frequency by the age of 18 – 24 months (*Nikitin, 2004*).

Table 2.6.5.1-7: Survival data: number of survived rats (% survival); (*Anonymous, 1988c)*

	0 ppm		**300 ppm**		**3000 ppm**		**6000 ppm**		**10000 ppm**	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
total number	50	50	50	50	50	50	50	50	50	50
week 52	48	46	49	49	50	49	49	47	47	50
week 80	37	38	38	40	36	45	35	38	34	45
week 92	31	31	30	33	31	37	27	31	24	38
week 104	22/50 (44%)	24/50 (48%)	25/50 (50%)	20/50 (40%)	25/50 (50%)	26/50 (52%)	17/50 (34%)	22/50 (44%)	18/50 (36%)	21/50 (38%)
week 105	21/50 (42%)	23/50 (46%)	24/50 (48%)	19/50 (38%)	24/50 (48%)	25/50 (50%)	17/50 (34%)	21/50 (42%)	15/50 (30%)	19/50 (38%)

Table 2.6.5.1-8: Incidence of macroscopic masses/nodules in the lungs; ( ) = number of examined animals, DOS = died on study, SAC = scheduled sacrifice

	0 ppm		**300 ppm**		**3000 ppm**		**6000 ppm**		**10000 ppm**	
	DOS	**SAC**	**DOS**	**SAC**	**DOS**	**SAC**	**DOS**	**SAC**	**DOS**	**SAC**
Males	(29)	(21)	(26)	(24)	(26)	(24)	(33)	(17)	(35)	(15)
	5	6	6	10	4	15	12	14	13	6
Females	(27)	(23)	(31)	(19)	(25)	(25)	(29)	(21)	(31)	(19)
	3	4	10	7	4	8	7	3	4	7

Table 2.6.5.1-9: Tumour analysis; *p<0.05, **p<0.01, Fisher exact test; terminal rates = observed tumour incidence at terminal kill (including animals dying or sacrificed in extremis during week(s) of terminal kill); overall rates = number of tumour bearing animals/number of animals examined at site;

Tumour analysis - males (ppm)					
	0	**300**	**3000**	**6000**	**10 000**
LIVER - hepatocellular adenoma					
overall rates	4/50 (8.0%)	4/50 (8.0%)	3/50 (6.0%)	4/50 (8.0%)	5/50 (10.0%)
terminal rates	2/21 (9.5%)	4/24 (16.7%)	3/24 (12.5%)	3/17 (17.6%)	2/15 (13.3%)
LIVER - haemangioma					
overall rates	2/50 (4.0%)	0/50 (0.0%)	2/50 (4.0%)	1/50 (2.0%)	2/50 (4.0%)
terminal rates	0/21 (0.0%)	0/24 (0.0%)	1/24 (4.2%)	0/17 (0.0%)	0/15 (0.0%)
LIVER - haemangiosarcoma					
overall rates	3/50 (6.0%)	1/50 (2.0%)	0/50 (0.0%)	2/50 (4.0%)	7/50 (14.0%)
terminal rates	0/21 (0.0%)	0/24 (0.0%)	0/24 (0.0%)	0/17 (0.0%)	0/15 (0.0%)
LIVER - hepatocellular carcinoma					
overall rates	4/50 (8.0%)	9/50 (18.0%)	7/50 (14.0%)	5/50 (10.0%)	2/50 (4.0%)
terminal rates	2/21 (9.5%)	3/24 (12.5%)	4/24 (16.7%)	2/17 (11.8%)	1/15 (6.7%)
LUNG - alveolar bronchiolar adenoma					
overall rates	20/50 (40.0%)	26/50 (52.0%)	28/50 (56.0%)	31/50 (62.0%)*	27/50 (54.0%)
terminal rates	9/21 (42.9%)	13/24 (54.2%)	18/24 (75.0%)	13/17 (76.5%)	8/15 (53.3%)
LUNG - alveolar bronchiolar carcinoma					
overall rates	5/50 (10.0%)	2/50 (4.0%)	5/50 (10.0%)	7/50 (14.0%)	6/50 (12.0%)
terminal rates	2/21 (9.5%)	1/24 (4.2%)	4/24 (16.7%)	3/17 (17.6%)	0/15 (0.0%)
LUNG - alveolar bronchiolar adenoma/carcinoma					

overall rates	25/50 (50.0%)	28/50 (56.0%)	33/50 (66.0%)	38/50 (76.0%)**	33/50 (66.0%)
terminal rates	11/21 (52.4%)	14/24 (58.3%)	22/24 (91.7%)	16/17 (94.1%)	8/15 (53.3%)
LIVER - haemangioma/haemangiosarcoma					
overall rates	5/50 (10.0%)	1/50 (2.0%)	2/50 (4.0%)	3/50 (6.0%)	9/50 (18.0%)
terminal rates	0/21 (0.0%)	0/24 (0.0%)	1/24 (4.2%)	0/17 (0.0%)	0/15 (0.0%)
Tumour analysis - females (ppm)					
	0	**300**	**3000**	**6000**	**10 000**
LIVER - hepatocellular adenoma					
overall rates	2/50 (4.0%)	0/50 (0.0%)	1/50 (2.0%)	2/50 (4.0%)	3/50 (6.0%)
terminal rates	1/23 (4.3%)	0/19 (0.0%)	0/26 (0.0%)	1/22 (4.5%)	2/20 (10.0%)
LIVER - haemangiosarcoma					
overall rates	1/50 (2.0%)	1/50 (2.0%)	1/50 (2.0%)	0/50 (0.0%)	3/50 (6.0%)
terminal rates	1/23 (4.3%)	0/19 (0.0%)	0/26 (0.0%)	0/22 (0.0%)	1/20 (5.0%)
LUNG - alveolar bronchiolar adenoma					
overall rates	20/50 (40.0%)	26/50 (52.0%)	27/50 (54.0%)	28/50 (56.0%)	26/50 (52.0%)
terminal rates	12/23 (52.2%)	12/19 (63.2%)	16/26 (61.5%)	17/22 (77.3%)	13/20 (65.0%)
LUNG - alveolar bronchiolar carcinoma					
overall rates	0/50 (0.0%)	3/50 (6.0%)	2/50 (4.0%)	2/50 (4.0%)	4/50 (8.0%)
terminal rates	0/23 (0.0%)	0/19 (0.0%)	0/26 (0.0%)	0/22 (0.0%)	0/20 (0.0%)
UTERUS - haemangiosarcoma					
overall rates	1/50 (2.0%)	0/42 (0.0%)	0/36 (0.0%)	0/41 (0.0%)	4/50 (8.0%)
terminal rates	0/23 (0.0%)	0/11 (0.0%)	0/12 (0.0%)	0/13 (0.0%)	1/20 (8.0%)
LUNG - alveolar bronchiolar adenoma/carcinoma					
overall rates	20/50 (40.0%)	29/50 (58.0%)	29/50 (58.0%)	30/50 (60.0%)*	30/50 (60.0%)*
terminal rates	12/23 (52.0%)	12/19 (63.2%)	16/26 (61.5%)	17/22 (77.3%)	13/20 (65.0%)
LIVER - haemangioma/haemangiosarcoma					
overall rates	3/50 (6.0%)	1/50 (2.0%)	2/50 (4.0%)	1/50 (2.0%)	4/50 (8.0%)
terminal rates	2/23 (8.7%)	0/19 (0.0%)	1/26 (3.8%)	0/22 (0.0%)	2/20 (10.0%)

Table 2.6.5.1-10: Historical control data for alveolar/bronchiolar adenomas and carcinomas (provided by the applicant; 4 experiments performed in the same laboratory as the mouse carcinogenicity study with daminozide *(Anonymous, 1988c)* during years 1983-1985)

	Males		**Females**	
Study	**Adenomas (%)**	**Carcinomas (%)**	**Adenomas (%)**	**Carcinomas (%)**
A	18.2	0.9	12.7	4.5
B	44.0	0.0	22.0	2.0
C	28.0	6.0	14.0	2.0
D	26.1	8.7	8.7	2.9

Table 2.6.5.1-11: Literature data on the spontaneoius incidence of alveolar/bronchiolar adenomas in CD-1 mice; *= Giknis (2005) extended by further studies;

Mouse strain	**Study**	**Rate**	**Range**	**Years**	**Relevance/ reliability**	**Reference**
Crl:CD-1 (ICR)BR	78-104 week studies	**Adenomas** ♂: 368/2575 (14.29%) ♀: 236/2773 (8.51%) **Carcinomas** ♂: 177/2575 (6.87%) ♀: 113/2773 (4.08%)	**Adenomas** ♂: 2-42% ♀: 1.67-26.67% **Carcinomas** ♂: 1.43-26% ♀: 0.77-18.37%	studies initiated between 1987-1996	**Lower**: some of the studies lasted only 78 weeks and were performed more than 5 years later than the study with daminozide, i.e. 1985 – 1987	*Hayes, 2014 (Textbook of toxicology; source Giknis, 2000)*
CD-1 mice	2-year studies	**Adenomas** ♂: 130/891 (14.6%) ♀: 129/890 (14.5%) **Carcinomas** ♂: 168/891 (18.9%) ♀: 108/890 (12.1%)	-**Data not available** ***see Table 2.6.5.1-12 for further information***	11 studies performed between 1983-1988	**Lower:** the same rat strain used; performed in time period as the study with daminozide (within ±5 years); the incidence of carcinomas seems to be too high; **the range for adenomas and carcinomas separately was not published**	*Hayes, 2014 (Textbook of toxicology; source Maita, 1988)*
Crl:CD-1 (ICR)BR	78-104 week studies	**Adenomas** ♂: 421/2945 (14.3%) ♀: 299/3143 (9.51%)	**Adenomas** ♂: 2-42% ♀: 1.67-26.67%	studies initiated between 1987-2000 (51 studies in males, 49 in	**Lower**: some of the studies lasted only 78 weeks and were performed more than 5 years later than the study with	*Giknis, 2005**

		Carcinomas ♂: 217/2945 (7.37%) ♀: 145/3143 (4.61%)	**Carcinomas** ♂: 1.43-26% ♀: 0.77-18.37%	females)	daminozide, i.e. 1985 – 1987	
CD-1 mice	2-year studies	**Adenomas** ♂: 19.3% ♀: 12.3% **Carcinomas** ♂: 2.5% ♀: 1.5%	**Data not available**	studies finished 1992; 725 males and females	**Lower:** the same rat strain used; performed in time period as the study with daminozide (within ±5 years); **the range was not published**	*Chandra, 1992*

Table 2.6.5.1-12: Range of incidence of alveolar/bronchiolar adenomas combined with carcinomas in CD-1 mice retrieved from open literature *(Maita, 1984)*; * = statistically significant variability ($p<0.05$)

Study	Males	Females
1	23/80 (28.8%)	16/80 (20%)
2	29/80 (36.3%)	20/79 (25.3%)
3	26/79 (32.9%)	26/80 (32.5%)
4	21/80 (26.3%)	14/80 (17.5%)*
5	28/80 (35.0%)	31/80 (38.8%)*
6	26/80 (32.5%)	18/80 (22.5%)
7	17/80 (21.3%)	22/80 (27.5%)
8	35/80 (43.8%)	27/79 (34.2%)
9	26/80 (32.5%)	19/80 (23.8%)
10	27/80 (33.8%)	19/80 (23.8%)
11	40/92 (43.5%)	25/92 (27.2%)
Total	298/891 (33.4%)	237/890 (26.6%)

Taking into account all these considerations, the rise in the incidence of pituitary adenomas in rats and pulmonary neoplasms in mice is regarded by RMS as the treatment-related, indicating oncogenic potential of daminozide, and relevant to humans. This conclusion is also supported by the occurrence of renal cell adenoma in one female during 1- year dietary toxicity study in dogs (*Anonymous, 1988a; see 2.6.3.1.1*). This type of neoplasm is rare with higher sensitivity in males (0.3 – 1.5% *Meuten, 2012*).

The mechanistic studies that might reveal the mechanism of carcinogenicity (mode of action) are not available. Based

on the negative studies on genotoxicity, the non-genotoxic mechanisms are supposed to be involved.

2.6.5.2 Comparison with the CLP criteria regarding carcinogenicity

According to CLP criteria (Regulation (EC) No. 1272/2008), classification in Category 1B (presumed human carcinogen) is based on animal experiments for which there is sufficient evidence to demonstrate carcinogenicity. The sufficient evidence of carcinogenicity means: a causal relationship between the agent and increased incidence of malignant neoplasms or an appropriate combination of benign and malignant neoplasms in (i) two or more species of animals or (ii) two or more independent studies in one species carried out at different times or in different laboratories or under different protocols. An increased incidence of tumours in both sexes of a single species in a well-conducted study can also provide sufficient evidence. A single study in one species and sex might be considered to provide sufficient evidence of carcinogenicity when malignant neoplasms occur to an unusual degree with regard to incidence, site, type of tumour or age at onset, or when there are strong findings of tumours at multiple sites.

The relationship between daminozide administration and increased incidence of neoplasms was found in two species of animals (pituitary adenoma in female rats and pulmonary tumours in both sexes of mice). Therefore, the proposed classification for daminozide is Carcinogen 1B (Category 1B). The classification Carcinogen 1A (Category 1A) is not applicable since no data on the carcinogenicity of daminozide in humans are available.

According to CLP criteria (Regulation (EC) No. 1272/2008), classification in Category 2 (suspected human carcinogen) is based on the evidence obtained from human and/or animal studies, but which is not sufficiently convincing to place the substance in Category 1A or 1B (i.e limited evidence). The limited evidence of carcinogenicity means that the data suggest a carcinogenic effect but are limited for making a definitive evaluation because, e.g. (a) the evidence of carcinogenicity is restricted to a single experiment; (b) there are unresolved questions regarding the adequacy of the design, conduct or interpretation of the studies; (c) the agent increases the incidence only of benign neoplasms or lesions of uncertain neoplastic potential; or (d) the evidence of carcinogenicity is restricted to studies that demonstrate only promoting activity in a narrow range of tissues or organs. In rats, daminozide increased only the incidence of pituitary adenomas, i.e. benign tumours, however, occurrence of pituitary carcinomas is in general rare (0%: in males as well as females, *Sandusky (1988)*, HCD of higher relevance and reliability used in the *Table 2.6.5.1-5*) . In mice, the incidence of both alveolar/bronchiolar adenomas and carcinomas was increased. Thus, in our opinion, daminozide does not meet criteria for classification in Category2 (suspected human carcinogen).

The major metabolite of daminozide, UDMH, exerted carcinogenic potential in animal studies (*see 2.6.8.1*). According to CLP criteria (Regulation (EC) No. 1272/2008), UDMH is classified as Carcinogen 1B (Category 1B).

ANNEX 1 – BACKGROUND DOCUMENT TO RAC OPINION ON DAMINOZIDE (ISO); 4-(2,2-DIMETHYLHYDRAZINO)-4-OXOBUTANOIC ACID; N-DIMETHYLAMINOSUCCINAMIC ACID

Table 44: Compilation of factors to be taken into consideration in the hazard assessment

Species and strain	**Tumour type and background incidence**	**Multi-site response**	**Progression of lesions to malignancy**	**Reduced tumour latency**	**Response in single or both sexes**	**Confounding effect by excessive toxicity?**	**Route of exposure**	**MoA and relevance to humans**
F344 Rats	Pituitary adenoma Concurrent control: ♂ 41.7%, ♀ 37.3% Literature data (*see Table 2.6.5.1-5 and 2.6.5.1-6)*: ♂ 25%, ♀ 42%, *Hayes (2014)*; ♂ 26%, ♀ 36%, *Sandusky (1988)*; ♂ 21.7%, ♀ 44%, *Haseman (19844)*;	No	Progression to carcinoma was not observed; in general pituitary carcinoma is very rare	**No**: during the time period 0 – 12 months (2-year carcinogenicity study), tumour incidence was not higher in treated groups than controls; during 90-day subchronic study, no pituitary adenoma was observed UDMH: **Yes** (see comments under the table)	Only in females	Excessive toxicity was not observed	The oral route is relevant	MoA is non-genotoxic; not elucidated in detail Relevant to humans (*see comments under the table*)
CD-1 Mice	Alveolar/bronchiolar adenoma and carcinoma Concurrent control: adenoma: ♂ 40%, ♀ 40% carcinoma: ♂ 10%, ♀ 0% Historical control	No	Adenomas progressed to carcinomas In females carcinomas were found only in treated animals (not in controls),	**Impossible to evaluate**: Incidence of tumours at the time period 0 – 12 months (2-year carcinogenicity study) was not stated; 90-day subchronic	In both sexes	Excessive toxicity was not observed	The oral route is relevant	MoA is non-genotoxic; not elucidated in detail Relevant to humas (*see comments under the*

Species and strain	Tumour type and background incidence	Multi-site response	Progression of lesions to malignancy	Reduced tumour latency	Response in single or both sexes	Confounding effect by excessive toxicity?	Route of exposure	MoA and relevance to humans
	(see Table 2.6.5.1-10): adenoma: ♀ 8.7-22%, ♂ 18.2-44% carcinoma: ♀ 2.0-4.5%, ♂ 0-8.7% Literature data *(see Table 2.6.5.1-11* and *2.6.5.1-12)*: adenoma: ♂ 2 – 42%, ♀ 2 – 27%, *Giknis (2005), Hayes (2014);* ♂ 14.6%, ♀ 14.5%, *Maita (2014);* ♂ 19.3%, ♀ 12.3%, *Chandra (1992);* carcinoma: ♂ 2.5%, ♀ 1.5%, *Chandra, (1992)*		which was not confirmed in males	study was not performed in mice; In females carcinomas were found only in treated animals (not in controls), which was not confirmed in males UDMH: **Yes** (see comments under the table)				*table*)

2.6.5.3 Conclusion on classification and labelling for carcinogenicity

<u>Reduced tumour latency</u>:

Effect of daminozide in rats: Based on the results of the 90-day subchronic and carcinogenicity studies in rats, it can be concluded that daminozide does not reduce the latency of pituitary adenomas.

Effect of daminozide in mice: Based on the available data, it is not possible to evaluate, whether daminozide could reduce the latency of alveolar/bronchiolar adenomas in mice because the 90-day mouse subchronic study was not conducted, and the incidence of tumours in the first year of 2-year carcinogenicity study was not stated in the original study report. Alveolar/bronchiolar carcinomas were observed during the 2-year carcinogenicity study only in treated females and not in controls. This might indicate that daminozide reduced the latency of alveolar/bronchiolar carcinomas and supported the progression of adenomas to carcinomas. However, in males, these effects were not confirmed as the

concurrent control was high *(see Table 2.6.5.1-9)*.

Effect of UDMH: The rat 2-year carcinogenicity study with UDMH *(see 2.6.8.1)*, however, revealed that the incidence of pituitary adenomas in females at the two highest doses (50 and 100 ppm) was increased already during the time period 0 – 12 months comparing to the control group. Thus, UDMH, the major metabolite of daminozide, might lower the latency of pituitary adenomas in rats. This effect of UDMH was also shown in case of alveolar/bronchiolar adenomas in mice, which were already observed in treated groups of both sexes during the 90-day subchronic study (*see 2.6.8.1*).

Mode of action and relevance to humans: Based on the results of genotoxicity studies, daminozide acts as a non-genotoxic carcinogen. According to *Guidance on the application of the CLP criteria*, *Guidance on information requirements and chemical safety assessment (Chapter R.7a)*, as well as Toxicology textbook *(Hayes, 2014)*, pituitary adenomas as well as alveolar/bronchiolar adenomas/carcinomas are not included in the list of tumours irrelevant for humans.

Pituitary adenomas: It is well-known that the majority of pituitary adenomas in both rodents and humans arise from prolactin-producing cells. The increased incidence of pituitary adenomas in older rats could be explained by the fact that the content of dopamine, which inhibits prolactin secretion, decreases with age *(Prysor-Jones, 1983)*. However, in the study with daminozide, the pituitary adenomas incidence of female controls correlated with literature data, whereas it was increased at each test dose *(see 2.6.5.1 for more information)*. And there is no reference that daminozide could act as neuroleptics, i.e. dopamine inhibitors.

Non-genetic methods to generate animal models of pituitary adenoma were developed, e.g. long-term treatment of ovariectomised F344 rats with oestrogen or injection of agents able to mimic oestrogens *(Lines, 2016)*. As for daminozide, mechanistic studies evaluating the possibility of the test substance to modify the endocrine system were not provided. However, neither results of short-term, long-term, and reproduction studies nor the literature data gave evidence that daminozide directly interferes with the function of the sexual, or thyroid hormone pathways *(see 2.6.8.3)*.

To date, the involvement of hormonal factors in pituitary tumorigenesis in humans is not fully understood. Pituitary adenomas secreting prolactin (prolactinomas) were reported in male-to-female transsexuals treated with oestrogens to induce the breast development or in girls treated with oestrogens to retard the excessive growth *(Gooren, 1988; Garcia, 1995; reviewed in Spady, 1999)*. The literature data on the relationship between the taking of contraceptive pills and risk of pituitary adenoma development are rather contradictory. Some studies claim that a causal link between the contraception usage and pituitary adenoma incidence was not established *(Babichev, 2001)*. Other studies revealed that women using the oral oestrogen contraceptive show higher prolactin levels as well as incidence of prolactinomas *(Luciano, 1985; Carol, 1988; Shy, 1983; reviewed in Sarkar, 2006)*. The recent study *(Benson, 2014)* proved that the menopausal oestrogen-only therapy, but not combined oestrogen – progestin therapy, increases the risk of CNS tumours including pituitary adenoma. These results indicate that the increased risk of pituitary adenomas might also depend on the kind of the used contraception, not only the menopausal hormone replacement therapy. Finally, it was shown that certain part of human population is more sensitive to oestrogen effects *(Laccarino, 2002; Oomizu, 2004)*, (similarly to rat strains), and thus might be in higher risk of pituitary adenoma development *(Sarkar, 2006)*.

Alveolar/bronchiolar adenomas/carcinomas: Since mechanistic investigations (e.g. *in vitro* metabolism study with lung microsomes, proliferation of lungs cell) to clarify the mode of non-genotoxic carcinogenic action of daminozide were not provided, it is not possible to come to a conclusion on the mechanisms involved.

Taken together, based on the available data, the mode of non-genotoxic carcinogenic action of daminozide cannot be

elucidated neither for pituitary adenomas nor alveolar/bronchiolar adenomas/carcinomas, and the relevance to humans cannot be excluded.

Proposed classification for daminozide: Carcinogen 1B (Category 1B).

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

The DS proposed classification of daminozide as Carc. 1B (presumed human carcinogen) based on sufficient evidence demonstrating carcinogenicity in animal studies. Chronic administration of daminozide led to an increased incidence of neoplasms in two different species: pituitary adenoma in female rats and pulmonary tumours in both sexes of mice. There were no mechanistic investigations to conclude on the possible mode of action for daminozide, and therefore the relevance to humans cannot be excluded. In addition, UDMH, the major metabolite of daminozide, is also carcinogenic in animal studies and classified as carcinogen of Category 1B in Annex VI of the CLP Regulation. Increased incidence of pituitary adenomas in female rats was reported at the two highest doses (50 and 100 ppm) from the 2-year carcinogenicity study with UDMH, and alveolar/bronchiolar adenomas in mice were observed in treated groups of both sexes during the 90-day subchronic study.

Comments received during public consultation

Five MSCAs and one manufacturer provided comments on this endpoint. One MSCA supported the DS proposal, two MSCAs discussed different aspects of the proposal expressing views on both Category 1B and Category 2. One MSCA clearly supported Category 2. After an *ad hoc* consultation of additional information, one of the MSCAs who earlier presented views on both 1B and 2, sent further comments to support Category 1B rather than Category 2.

One MSCA considered that the contribution to the carcinogenic potential of metabolites (e.g. UDMH) should also be included in the evaluation of whether daminozide fulfils the requirement for classification for carcinogenicity, and if yes, in which category.

One MSCA considered that support for Carc. 1B can be given since it cannot be excluded that the formation of UDMH and its further conversion into NDMA does not occur in humans as well. One MSCA pointed out that other neoplasms in 2-year mouse study showed increased incidences in the treated groups and it would be interesting to compare their incidences with relevant laboratory historical control data (HCD), e.g. hemangiosarcomas in the uterus and liver (also observed in the study with UDMH). Furthermore, it should be noted that NTP studies were conducted on daminozide (NCI Carcinogenesis technical report Series No 83). In this study, hepatocellular carcinomas were observed in male mice whereas adenocarcinomas of the uterine endometrium and leiomyosarcomas of the uterus were observed in female rats.

The manufacturer was in favour of no classification arguing that there is no evidence of carcinogenicity in either rats or mice. The pulmonary tumours in mice were a common tumour in the strain used, and the observed rates were without a dose relationship despite substantial dose spacing. The neoplastic effect in the rat study could be explained with the specific tumour analysis on a selectively chosen incomplete data set.

One downstream user commented during the *ad hoc* consultation, suggesting classification for Category 2 based on a statistically significant increase in the overall incidence of pituitary adenoma in the female rat and of pulmonary tumours in male and female mice.

Assessment and comparison with the classification criteria

2-year carcinogenicity study in rats (Anonymous, 1988b)

In a combined chronic toxicity/carcinogenicity study (OECD TG 453, EPA OPP 83-2), Fischer 344 rats (60 animals/sex/dose; interim sacrifice of 10 sex/dose after one year) were exposed to daminozide (purity 99%) via food at doses of 0, 100, 500, 5000 and 10000 ppm (corresponding to 0, 5, 25, 250, 500 mg/kg bw/day), for 24 months. Reported deviations from the current guideline include lacking investigations of some haematological parameters, and missing weights for epididymides, uterus, and thyroid at necropsy after the chronic toxicity phase. The number of animals survived at week 104 was similar among all groups including controls (52%-80%), with higher mortality for males than for females.

No treatment-related clinical signs, effect on ophthalmoscopy, haematology, biochemistry, and urinalysis were observed during the study. Macroscopic lesions observed at necropsy included cloudy corneas in the eyes, granular kidneys/livers, coloured foci and/or enlarged pituitary, enlarged spleens, nodules or masses in the uterus and coloured foci in the testes.

Several microscopic lesions such as chronic progressive nephropathy in males, single incidences of haemolytic anaemia, and testicular interstitial cell tumours were reported in the study without obvious dose response and considered chance findings. Higher incidences of ovarian atrophy and ovarian cysts, and hepatic bile duct hyperplasia were reported in treated females. Although without clear dose response, these findings may have been treatment related and were addressed in STOT RE section (Table below).

***Table**: Summary of some non-neoplastic histopathological findings from the chronic toxicity and carcinogenicity study with daminozide (Anonymous, 1988b)*

Dose ppm (mg/kg bw/day)	**0 ppm (0)**		**100 ppm (5)**		**500 ppm (25)**		**5000 ppm (250)**		**10000 ppm (500)**	
Time of examination	DOS	SAC	DOS	SAC	DOS	SAC	DOS	SAC	DOS	SAC
Males	(25)	(24)	(26)	(23)	(18)	(32)	(24)	(26)	(12)	(37)
Bile duct hyperplasia	17	19	17	16	11	27	18	21	10	31
Females	(15)	(35)	(12)	(37)	(13)	(37)	(11)	(39)	(15)	(35)
Bile duct hyperplasia	1	4	3	13	2	11	6	10	2	12
Ovarian atrophy	2	0	4	1	6	3	2	3	7	3
Ovarian cysts	2	0	0	5	2	4	2	5	2	6

( )=number of examined animals, DOS=died on study, SAC=scheduled sacrifice

A single incidence of adamantinoma (a rare oral neoplasm) occurred in one male of the high dose group during the first 12 months of the study, however no other significant macroscopic oral pathology was observed in treated groups. Thus, the study author considered the occurrence of this tumour as an incidental finding.

A statistically significant increase in the incidence of pituitary adenomas was observed in female rats starting from the lowest test group (excluding top dose) and regarded by DS as a treatment-related effect (Table below).

***Table**: Tumour analysis of pituitary adenoma in male and female F344 rats (Anonymous, 1988b)*

Dose ppm (mg/kg bw/day)	**0 ppm (0)**	**100 ppm (5)**	**500 ppm (25)**	**5000 ppm (50)**	**10000 ppm (500)**
Males					
Overall rates (%)	25/60 (41.7)	12/31 (38.7)	17/27 (63.0)	12/27 (44.4)	27/59 (45.8)
Terminal rates (%)	11/25 (44.0)	4/7 (57.1)	9/10 (90.0)	3/3 (100)	19/36 (52.8)
Females					
Overall rates (%)	22/59 (37.3)	18/25** (72.0)	27/32*** (84.4)	19/25*** (76.0)	27/58 (46.6)
Terminal rates (%)	16/36 (44.4)	12/12 (100)	18/18 (100)	13/13 (100)	20/36 (55.6)

*p<0.05, **p<0.01, ***p<0.001; Terminal rates=observed tumour incidence at terminal kill (including animals dying or sacrificed in extremis during week(s) of terminal kill); Overall rates=number of tumour bearing animals/number of animals examined at site

In its initial assessment, the DS considered these tumours treatment-related while acknowledging that F344 rats are rather susceptible to developing pituitary adenomas, with literature data pointing to average spontaneous rates of 37% - 49% in females (Haseman *et al.*, 1984).

***Table**: Inter-laboratory variability in literature data on the spontaneous incidence of pituitary adenomas in Fischer 344 rat (Haseman et al., 1984)*

Laboratory	**No of studies /No of males**	**Rate**	**Range**	**No of studies/ No of females**	**Rate**	**Range**
A	9/439	17%	5-29%	9/439	37%	18-50%
B	5/249	18%	6-28%	5/249	45%	42-52%
C	14/699	24%	7-52%	15/747	49%	30-70%
D	6/340	18%	8-41%	6/337	45%	30-64%
E	7/344	30%	19-44%	7/350	42%	26-67%

The relevance and reliability of these literature data are in general rather low, since they do not resemble historical controls from the performing laboratory as specified in the guidance. Relevant HCD for female rats from the performing laboratory (six studies initiated from 1979-1982) submitted subsequently indicate spontaneous rates of 23.2% - 40.0% that are in line with the literature data (Table below).

***Table**: Historical control data on pituitary adenoma in female Fischer 344 rats obtained during several 2-year chronic studies from the performing laboratory*

Route	**Gavage**	**Dietary**	**IV**	**Dietary**	**Dietary**	**Dietary**
Study duration	7/79-7/81	10/80-10/82	4/81-5/83	11/81-11/83	9/82-9/84	12/82-12/84
Initial number	60	50	50	80	70	60
Incidence (%)	16/59 (27.1)	14/50 (28)	12/50 (24)	32/80 (40)	25/70 (35.7)	13/56 (23.2)

Notably, increased incidence of pituitary adenoma was also observed in the rat carcinogenicity study with the major daminozide metabolite UDMH.

During PC, the applicant provided additional details on the study design commenting that a complete histological examination at terminal sacrifice was performed for all animals only in the control and high dose groups, while at mid-doses (100, 500, and 5000 ppm) only animals with macroscopic abnormalities and those that died during the study were examined. Since pituitary tumours are easy to macroscopically detect at necropsy (swelling 5 or 10 times the normal size), it seems that only animals with these abnormalities were selected for further histopathology examination leading therefore to misleading incidence rates at these doses. In line with this argumentation, the increased incidence of pituitary adenomas at the highest dose was not statistically significant when compared to control, and the rate of 46.6 % was slightly above the upper range of HCD from the performing laboratory (40%). In view of these clarifications, the DS revised the CLH report accordingly and regarded the incidence of pituitary adenomas in females as not treatment-related effect.

Considering the additional information provided during PC and the revised CLH report, RAC supports the views of DS and the applicant that it would be incorrect to attempt a tumour analysis on an incomplete data set, which by its design focused selectively on animals showing abnormalities. Therefore, RAC concludes that classification for carcinogenicity based on reported incidences of pituitary adenomas in female rats is not supported.

2-year carcinogenicity study in mice (Anonymous, 1988c)

Groups of 50 male and 50 female CD-1 mice were given Alar® Technical (daminozide, purity 99%) in the diet at concentrations of 0, 300, 3000, 6000 and 10000 ppm (corresponding to 0, 45, 450, 900, and 1500 mg/kg bw/day) for 24 months. The study was performed according to OECD TG 451 without reported deviations. The survival of females at the end of the study was slightly decreased at the high dose, and in males of the mid and high dose (Table below). No effects on body weight and food consumption were reported.

***Table**: Survival data (n=50/sex/group) (Anonymous, 1988c)*

Dose ppm (mg/kg bw/day)	**0 ppm (0)**		**300 ppm (45)**		**3000 ppm (450)**		**6000 ppm (900)**		**10000 ppm (1500)**	
Sex	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
week 52	48	46	49	49	50	49	49	47	47	50
week 104	22/50 (44%)	24/50 (48%)	25/50 (50%)	20/50 (40%)	25/50 (50%)	26/50 (52%)	17/50 (34%)	22/50 (44%)	18/50 (36%)	21/50 (38%)

Females at the highest dose group showed significantly decreased erythrocyte count, and inflammation as well as brown pigmentation in the liver was noted in males. A statistically significant decrease in the mean platelet count was observed in females at ≥3000 ppm.

A full set of organs and tissues was microscopically examined for all animals in the control and high dose groups, as well as for those that died during the study. Sections were also prepared for target organs (kidney, liver and lung) and detected gross lesions in the low and mid dose groups. Additional serial sections were prepared for the lungs of animals without microscopically detectable lung tumours.

An increased incidence of nodules/masses was observed in the lungs of the treated mice of both sexes. These findings did correlate with some of the microscopically-detected neoplasms; however, there were also a large number of pulmonary neoplasms that were

undetectable grossly.

An increased incidence of alveolar/bronchiolar adenomas as well as alveolar/bronchiolar adenomas combined with carcinomas was observed in all dose groups of both sexes when compared to control. Tumour analysis of adenomas or carcinomas alone revealed no statistical significance for treatment related effect. The increase in the tumour incidences compared to the control was statistically significant (Fisher exact test) only for adenomas and combined adenomas/carcinomas in males at 6000 ppm, and for combined adenomas/carcinomas in females at ≥6000 ppm (Table below).

***Table**: Lung tumour analysis: alveolar bronchiolar adenoma/carcinoma in male and female CD-1 mice (Anonymous, 1988c)*

Dose ppm	**0**	**300**	**3000**	**6000**	**10000**
Males					
LUNG - alveolar bronchiolar adenoma					
Overall rates (%)	20/50 (40.0)	26/50 (52.0)	28/50 (56.0)	31/50 (62.0)*	27/50 (54.0)
Terminal rates (%)	9/21 (42.9)	13/24 (54.2)	18/24 (75.0)	13/17 (76.5)	8/15 (53.3)
LUNG - alveolar bronchiolar carcinoma					
Overall rates (%)	5/50 (10.0)	2/50 (4.0)	5/50 (10.0)	7/50 (14.0)	6/50 (12.0)
Terminal rates (%)	2/21 (9.5)	1/24 (4.2)	4/24 (16.7)	3/17 (17.6)	0/15
LUNG - alveolar bronchiolar adenoma/carcinoma					
Overall rates (%)	25/50 (50.0)	28/50 (56.0)	33/50 (66.0)	38/50 (76.0)**	33/50 (66.0)
Terminal rates (%)	11/21 (52.4)	14/24 (58.3)	22/24 (91.7)	16/17 (94.1)	8/15 (53.3)
Females					
LUNG - alveolar bronchiolar adenoma					
Overall rates (%)	20/50 (40.0)	26/50 (52.0)	27/50 (54.0)	28/50 (56.0)	26/50 (52.0)
Terminal rates (%)	12/23 (52.2)	12/19 (63.2)	16/26 (61.5)	17/22 (77.3)	13/20 (65.0)
LUNG - alveolar bronchiolar carcinoma					
Overall rates (%)	0/50	3/50 (6.0)	2/50 (4.0)	2/50 (4.0)	4/50 (8.0)
Terminal rates (%)	0/23	0/19	0/26	0/22	0/20
LUNG - alveolar bronchiolar adenoma/carcinoma					
Overall rates (%)	20/50 (40.0)	29/50 (58.0)	29/50 (58.0)	30/50 (60.0)*	30/50 (60.0)*
Terminal rates (%)	12/23 (52.0)	12/19 (63.2)	16/26 (61.5)	17/22 (77.3)	13/20 (65.0)

*p<0.05, **p<0.01, Fisher exact test; terminal rates=observed tumour incidence at terminal kill (including animals dying or sacrificed in extremis during week(s) of terminal kill); overall rates=number of tumour-bearing animals/number of animals examined at site

The incidence of adenomas in males in the concurrent controls (40%) is within the range of

HCD provided by the applicant (15-44%, see Table below). The incidence of adenomas in females in the concurrent controls (40%) is outside the HCD (6-22%). Carcinomas in the concurrent controls in males (10%) and in females (0%) were within the range of HCD (0-15.8% for males, 0-6% for females). After administration of daminozide, the incidences of adenomas in both males and females and carcinomas in females were outside of the range of HCD.

***Table**: Historical control data for alveolar/bronchiolar adenomas and carcinomas provided by the applicant; studies performed in the same laboratory during years 1981-1985*

Study	**Males**		**Females**	
	Adenomas (%)	**Carcinomas (%)**	**Adenomas (%)**	**Carcinomas (%)**
A (7/81-7/83)	9/60 (15) 11/50 (22.0)	1/60 (1.7) 0/50	5/60 (8.3) 9/50 (18.0)	2/60 (3.3) 3/50 (6.0)
B (1/82-1/84)	22/50 (44.0)	0/50	11/50 (22.0)	1/50 (2.0)
C (10/82-9/84)	14/50 (28.0)	3/50 (6.0)	7/50 (14.0)	1/50 (2.0)
D (10/83-10/85)	11/50 (22.0) 7/19 (36.8)	3/50 (6.0) 3/19 (15.8)	3/50 (6.0) 3/19 (15.8)	2/50 (4.0) 0/19

According to DS, while known to be prone to lung neoplasia, CD-1 mice are considered less susceptible to alveolar/bronchiolar adenoma than other mouse strains such as A/J showing rates of up to 100% by the age of 18-24 months (Nikitin, 2004). Further, the unusually high incidence of adenomas in the concurrent control group (40%) is additionally complicating the interpretation of the results.

The available data do not allow to conclude whether daminozide could reduce the latency of alveolar/bronchiolar adenomas and carcinomas in mice because a 90-day study is not available, and the incidence of tumours in the first year of this study was not reported.

In males, there was also a positive trend in the life table tests both for liver haemangiosarcoma alone and for combined haemangiomas/haemangiosarcomas, however pairwise comparisons with the controls were not statistically significant for any treated groups (Table below). Therefore, this was not considered a biologically significant effect in this study.

***Table**: Liver vascular neoplasms: haemangioma and haemangioma/haemangiosarcoma in male and female CD-1 mice (Anonymous, 1988c)*

Dose (ppm)	**0**	**300**	**3000**	**6000**	**10000**
Males					
LIVER - haemangioma					
Overall rates (%)	2/50 (4.0)	0/50 (0.0)	2/50 (4.0)	1/50 (2.0)	2/50 (4.0)
Terminal rates (%)	0/21 (0.0)	0/24 (0.0)	1/24 (4.2)	0/17 (0.0)	0/15 (0.0)
LIVER - haemangiosarcoma					
Overall rates (%)	3/50 (6.0)	1/50 (2.0)	0/50 (0.0)	2/50 (4.0)	7/50 (14.0)
Terminal rates (%)	0/21 (0.0)	0/24 (0.0)	0/24 (0.0)	0/17 (0.0)	0/15 (0.0)
LIVER - haemangioma/haemangiosarcoma					

Overall rates (%)	5/50 (10.0)	1/50 (2.0)	2/50 (4.0)	3/50 (6.0)	9/50 (18.0)
Terminal rates (%)	0/21 (0.0)	0/24 (0.0)	1/24 (4.2)	0/17 (0.0)	0/15 (0.0)
Females					
LIVER - haemangiosarcoma					
Overall rates (%)	1/50 (2.0)	1/50 (2.0)	1/50 (2.0)	0/50 (0.0)	3/50 (6.0)
Terminal rates (%)	1/23 (4.3)	0/19 (0.0)	0/26 (0.0)	0/22 (0.0)	1/20 (5.0)
LIVER - haemangioma/haemangiosarcoma					
Overall rates (%)	3/50 (6.0)	1/50 (2.0)	2/50 (4.0)	1/50 (2.0)	4/50 (8.0)
Terminal rates (%)	2/23 (8.7)	0/19 (0.0)	1/26 (3.8)	0/22 (0.0)	2/20 (10.0)

RAC notes that haemangiomas are benign neoplasms whereas haemangiosarcomas are malignant neoplasms originating from endothelial cells lining blood vascular spaces. Spontaneous haemangiosarcomas arise frequently in mice (2–5%), less commonly in rats (0.1–2%), and frequently in numerous breeds of dogs (about 2%). In mice, the spontaneous incidence is higher in males than females, and higher in B6C3F1, CD-1, and BALB/c strains than CBA/J (reviewed in Cohen *et al.*, 2009). Limited HCD from the performing laboratory indicate spontaneous rates in male CD-1 mice between 2.0% and 7.7% (4 studies initiated between 07/1981 and 10/1983), while HCD collected in the period 1987-2000 are between 1.1% and 8.6% (out of relevant time window). Considering the lack of statistical significance in the pairwise comparison between the groups, RAC does not consider the increased incidence of liver haemangiosarcoma in top dose male mice as an evidence supporting classification for carcinogenicity of daminozide.

RAC further notes that the incidence of alveolar/bronchiolar adenoma in control male mice (40%) is within the range of the relevant historical control data from the testing laboratory (15-44%). The incidence rates for alveolar/bronchiolar adenoma and adenoma/carcinoma combined in male mice were statistically significant increased over the concurrent control only at 6000 but not at 10000 ppm, and did not show clear dose response. It is further noted that when only lung carcinoma in females is considered, there may have been a trend to slightly higher incidences over the controls, however without statistical significance. However, this apparent trend might be due to the rather very low incidence of alveolar/bronchiolar carcinoma in control female mice (0%) which is at the lower range of the HCD for this tumour type (0-6%). RAC considers the increased incidence of alveolar/bronchiolar adenoma and carcinoma in all dose groups as treatment-related and relevant for classification. Nevertheless, in light of the high susceptibility of CD-1 mice to alveolar/bronchiolar adenoma and the lack of clear dose response, these findings should be interpreted with caution.

Carcinogenicity bioassay of daminozide (Campbell et al. 1978)

Fischer 344 rats (50/dose/sex) and B6C3F1 mice (50/dose/sex) were administered daminozide (purity 99.2-100%) in the diet at doses of 0, 5000 and 10000 ppm for 104 weeks (20/sex in controls). The study is non-GLP compliant, and no particular guideline was followed. Due to the absence of food consumption data the exact chemical intake cannot be calculated; using standard conversion factors intakes would be approximately 250 or 500 mg/kg bw/day in rats, and 715 or 1430 mg/kg bw/day in mice. Feed preparations containing the test substance were stored at 1ºC for no longer than one week. The recovery of the test substance in feed decreased by about 20% after storage for ten days at 25ºC, indicating that daminozide in the diets may have not been stable under the conditions of this study. Further, some of the animals in the study (including 6/20 male mice of control) were unaccounted for,

and no individual data is reported except overall means for each exposure group. Overall, reliability of the study is considered rather low.

The mean body weight of rats was unaffected in any of the groups, while female mice of the high dose showed lower body weight than the matched controls. No clinical signs were reported for both rats and mice.

The most significant finding in rats was an increased incidence of malignant tumours of the uterus. Adenocarcinomas of the endometrium at rates of 0/19 (0%), 5/50 (10%), and 3/50 (6%) and leiomyosarcomas of the uterus at rates of 0/19 (0%), 1/50 (2%) and 3/50 (6%) were reported in females of controls, low and high dose groups, respectively. These incidences were not statistically significant, however, outside of the HCD of 2/220 (0.9%) for adenocarcinoma and 0/220 (0%) for leiomyosarcoma. It is noted that HCD at this early point of the NTP testing program was rather limited. Control data from all laboratories in the bioassay program compiled to date show incidence rates for adenocarcinomas of the uterus/endometrium of 4/1659 (0.24%) and for leiomyosarcomas of the uterus of 1/1659 (0.06%). Thus, the occurrence of these tumours in the dosed animals was considered associated with the administration of daminozide.

In male rats, a positive dose-related trend in the incidence of interstitial cell tumours of the testis was reported at rates of 13/20 (65%), 46/50 (92%), and 47/50 (94%), respectively (Cochran-Armitage test, p=0.003). The incidences in both the low and high dose groups were significantly higher than in the matched controls (Fisher exact test, p=0.009 and p=0.004, respectively), however mortality in the control males was higher from week 60 thus possibly affecting the statistical significance of these tumours in the dosed groups. Considering the high spontaneous rate of the interstitial-cell tumours in male rats (HCD of 182/220, 83%), a clear association of this tumour with the administration of daminozide cannot be established. Overall, the report concluded that daminozide was carcinogenic in female Fischer 344 rats.

***Table**: Summary of selected neoplastic findings in rats*

Dose (ppm)	**0**	**5000**	**10000**
Estimated dose in mg/kg bw d	0	250	500
Animals initially in the study	20	50	50
Males			
Necropsied animals	20	50	50
Interstitial cell tumours of testis	13 (65%)	46 (92%)	47 (94%)
Females			
Necropsied animals	19	50	50
Leiomyosarcoma, uterus	-	1 (2%)	3 (6%)
Adenocarcinoma, endometrium/uterus	-	5 (10%)	3 (6%)

In the male mice, there was a positive dose-related trend (Cochran-Armitage test, p=0.008) in the incidence of hepatocellular carcinomas (0/14 (0%), 7/50 (14%) and 13/46 (28%), respectively, and the incidence in the high-dose group was significantly higher than in the controls (Fisher exact test, p=0.020). Only three hepatocellular carcinomas occurred in female mice of the low dose, and no such tumours were observed in the high dose female mice or in the controls of either sex. The incidence of hepatocellular carcinomas in the female mice is not significant using either statistical test. HCD for male mice at the performing laboratory show a rate of 21/216 (10%), and HCD from the entire bioassay program show an incidence of 266/2182 (12.2%). Therefore, the liver tumour incidence in concurrent controls

appears unusually low for this strain. In addition, due to six unaccounted animals, the male control group consisted of only 14 mice. The high background incidence and therefore questionable reliability of liver tumour profiles in B6C3F1 mice is specifically addressed in the current CLP Guidance. The report concluded that a clear association of hepatocellular carcinomas and daminozide administration cannot be established.

In female mice, an increased incidence of alveolar/bronchiolar adenomas/carcinomas was observed (statistically not significant): 1/20 (5%), 8/50 (16%), 10/50 (20%) at 0, 5000, and 10000 ppm, respectively (HCD: 12/227, 5.5%). The increased incidence of this type of tumour was also found in the key mice carcinogenicity study with daminozide (Annonymous, 1988c).

***Table**: Summary of selected neoplastic findings in mice*

Dose (ppm)	**0**	**5000**	**10000**
Estimated dose in mg/kg bw d	0	715	1430
Animals initially in the study	20	50	50
Males			
Missing animals	6	-	4
Necropsied animals	14	50	46
Hepatocellular adenoma	1 (7%)	2 (4%)	1 (2%)
Hepatocellular carcinoma	-	7 (14%)	13 (28%)
Females			
Missing animals	-	9	2
Necropsied animals	20	41	48
Hepatocellular adenoma	1 (5%)	1 (3%)	
Hepatocellular carcinoma	-	3 (8%)	13 (28%)
Alveolar/bronchiolar adenoma	-	4 (10%)	8 (17%)
Alveolar/bronchiolar carcinoma	1 (5%)	4 (10%)	2 (4%)

Conclusion on classification for carcinogenicity

There are three animal studies available investigating the carcinogenic potential of daminozide. In a combined chronic toxicity and carcinogenicity study performed in accordance with OECD TG 453 (Anonymous, 1988b), an increased incidence of pituitary adenomas was reported in female Fischer 344 rats. The increase was statistically significant at low and intermediate doses, and exceeded the rates observed in concurrent controls and spontaneous tumour incidence from the performing laboratory. However, the high incidence of pituitary adenoma in these dose groups can be explained by the design of the study analysis where only animals with gross abnormalities in these groups were processed for further histopathological examination at terminal sacrifice. Therefore, these results do not provide a clear indication of carcinogenicity in rats.

In another guideline compliant carcinogenicity study (OECD TG 451), alveolar/bronchiolar adenomas and carcinomas were observed in both sexes in each treated group in CD1 mice (Anonymous, 1988c). The increased incidence of combined alveolar/bronchiolar adenoma and carcinoma was statistically significant in males of the mid dose and in females at mid and high doses, however without clear dose relationship. The lack of dose-response might have been possibly related to the slightly higher mortality rates at the top dose of ca. 1500 mg/kg bw/day. The DS considered this effect as treatment-related. In view of the high susceptibility of CD-1 mice to alveolar/bronchiolar adenoma (up to 44% and 15.8% in males and females,

respectively), and their high incidence in concurrent control (up to 50% for combined adenoma/carcinoma), the observed rates of pulmonary tumours in both sexes should be interpreted with caution. Notably, this type of tumour was also found in studies with the daminozide metabolite UDMH. Therefore, even without dose response, its significance in an overall weight of evidence approach cannot be excluded.

Earlier NTP studies conducted with daminozide reported increased incidences of adenocarcinomas of the uterine endometrium and leiomyosarcomas of the uterus in female rats and hepatocellular carcinomas in male mice (Campbell *et al.*, 1978). The study was neither GLP nor guideline compliant, and showed several deficiencies regarding stability of the test substance in diet preparations, lower animal numbers, and the lack of exact food consumption data. The increased incidence of hepatocellular carcinoma in male mice might have been at least partly related to the unusually low rates and animal numbers in the control group (only 14 males and 6 unaccounted for). In addition, B6C3F1 is known to have higher background rates of liver tumours compared to other mouse strains, therefore the reliability of this finding is questionable as specifically addressed in the current CLP Guidance. The reported incidences of endometrial adenocarcinoma (6-10%) and uterine leiomyosarcoma (6%) were not statistically significant increased over the concurrent controls, however outside of the HCD. Considering more recent HCD and assuming binomial distributions for these tumours, the DS estimates the probability of 3/50 leiomyosarcomas in high-dose animals and 5/50 adenocarcinomas in low-dose animals being a chance finding as less than 0.001. Uterine leiomyosarcoma is a rare and aggressive malignant tumour that arises from the smooth muscle lining the walls of the uterus (myometrium) and has a very poor overall prognosis in humans. The original report concluded that daminozide was carcinogenic in female Fischer 344 rats. While the occurrence of this tumour type in female rats can possibly be linked to the significant methodological deficiency of the study, it should not be completely disregarded in the overall carcinogenicity assessment of daminozide.

According to the CLP Regulation (Annex I: 3.6.2.2.4), additional considerations as part of a weight of evidence approach has to be taken into account for a classification for carcinogenicity. In the context of daminozide evaluation, these factors are briefly assessed below:

a) Tumour type and background incidence

Alveolar/bronchiolar adenomas and carcinomas are tumours of relatively high spontaneous incidence in rats and mice, while uterine adenocarcinomas/leiomyosarcomas are rare (and aggressive) type of malignancy. Both tumour types are considered relevant to humans.

b) Multi-site responses

Daminozide induces tumours in various tissues (lung, possibly uterus)

c) Progression of lesions to malignancy:

Malignant tumours were found in the lung and uterus

d) Reduced tumour latency:

No indication for reduced latency of both tumour types.

e) Whether responses are in single or both sexes:

Alveolar/bronchiolar adenomas and carcinomas were reported in mice of both sexes, while uterine adenocarcinomas/leiomyosarcomas were observed in female rats.

f) Whether responses are in a single species or several species:

Different types of tumours occurred in mice and in rats.

g) Structural similarity to a substance(s) for which there is good evidence of carcinogenicity:

No specific evidence. Formation of two carcinogenic metabolites, UDMH and NDMA, is reported in animal studies, and metabolism in humans cannot be excluded.

h) Routes of exposure:

The available carcinogenicity studies were performed by oral route. Data for other routes of exposure are not available.

i) Comparison of absorption, distribution, metabolism and excretion between test animals and humans:

No reliable information is available, similar pharmacokinetics are assumed.

j) The possibility of a confounding effect of excessive toxicity at test doses:

Malignancy was observed at dose levels without excessive toxicity.

k) Mode of action and its relevance for humans, such as cytotoxicity with growth stimulation, mitogenesis, immunosuppression, mutagenicity:

There are no specific investigations on mode of action, and daminozide was not mutagenic. The formation of the potentially carcinogenic metabolites UDMH and to less extent NDMA was demonstrated in animal studies.

Comparison with the CLP criteria

No human data was available. Thus, classification as Carc. 1A is not justified.

Animal data on carcinogenicity of daminozide is available from three chronic toxicity studies in rats and in mice. According to the CLP criteria, classification as Carc. 1B is based on establishing a causal relationship between exposure to the agent and an increased incidence of malignant neoplasms or of an appropriate combination of benign and malignant neoplasms in (a) two or more species of animals or (b) two or more independent studies in one species carried out at different times or in different laboratories or under different protocols. Significant increases in the combined incidences of alveolar/bronchiolar adenoma and carcinoma were observed in CD1 mice of both sexes, however without clear dose relationship. Considering the high incidence of these tumours in concurrent and historical controls, and the lack of clear dose response, the study does not provide a definitive support for a causal relationship between exposure and cancer development. Nevertheless, the study was performed according to the existing guidelines, and the data indicate a neoplastic potential in mice. In addition, the same type of tumour was also observed in chronic studies with the major metabolite UDMH indicating that the occurrence of this tumour type is not likely to be solely a chance finding. Increased incidences of adenocarcinomas of the uterine endometrium and leiomyosarcomas of the uterus in female rats were reported in an older NTP study. The rates of these tumours were not statistically significant increased over the concurrent controls, however clearly outside of the contemporary and to date HCD. Uterine leiomyosarcoma is a rare and aggressive malignancy of high relevance for humans, however the reported deficiencies of the study do not allow to establish a clear and unequivocal link between treatment and carcinogenic outcome. In an overall weight of evidence assessment,

the lung tumours reported in male and female mice as well as the uterine tumours in female rats are considered relevant for carcinogenicity classification. Daminozide was clearly not genotoxic, and the carcinogenicity profile of its major metabolite UDMH should be sufficiently covered by the *in vivo* data on the parent chemical.

According to CLP, placing of a substance in Category 2 is done on the basis of evidence which is not sufficiently convincing to place the substance in Category 1A or 1B, based on strength of evidence together with additional considerations. RAC therefore concludes that support for category 1B is not strong enough and considers **classification of daminozide as Carc. 2** to be more appropriate.

Supplemental information - In depth analyses by RAC

Genotoxicity and repeat dose toxicity studies were provided for the metabolite UDMH in order to aid the assessment of daminozide.

Genotoxicity

In vitro, UDMH was not mutagenic in bacteria or mammalian cells, either with or without metabolic activation (Stankowski, 1986). UDMH was also negative in an *in vitro* UDS assay in rat hepatocytes (Barfknecht, 1986). *In vitro* mammalian cell gene mutation assay (HPRT) with CHO cells showed equivocal (Stankowski, 1987) and subsequently negative results (Stankowski, 1988). The studies were GLP compliant, however not performed in accordance with OECD TG 476. *In vitro* chromosome aberration assay was negative, however no independent repeat was performed and information on the purity of the test substance was limited (San Sebastian, 1986).

No *in vivo* genotoxicity studies with UDMH were provided by the notifiers. From the open literature, UDMH was found positive in an *in vivo* micronucleus test in mouse liver cells (Cliet *et al.*, 1989). In this study, CD1/CR mice were treated twice (24 h between treatments) with doses of 0, 12, 24, and 58 mg/kg bw UDMH via intraperitoneal injections, and then 24 h after the second treatment subjected to partial hepatectomy (PH). The incidence of micronucleated hepatocytes was determined 96 h after PH. For each dose, 10000 hepatocytes were examined (2000/animal for treated group), with the corresponding number of micronucleated cells. UDMH showed a statistically significant increase in the frequency of micronucleated hepatocytes compared with controls (p≤0.01, Table below).

***Table**: Frequencies of micronucleated hepatocytes in CD1/CR mice treated with UDMH compared with controls*

	Group size	**Dose (mg/kg bw)**	**Micronucleated hepatocytes per 1000 cells**
Control (water)	20	0	3.3 ± 1.3
Control (methylcellulose)	10	0	3.9 ± 1.5
UDMH	5	14	7.1 ± 0.9*
	5	28	10.5 ± 0.9*
	5	56	8.4 ± 2.0*

*p≤0.01 (chi-square test)

The reliability of this study for genotoxicity assessment of UDMH has been criticized by the applicant regarding several deficiencies. These concerned specifically the endpoint micronuclei formation in hepatocytes as being insufficiently validated, the non-physiological route of

administration (i.p. injection), and the lack of purity and stability characterisation of the stock UDMH solutions. Nevertheless, the results were comparable with those of other known mutagens examined in the same study, i.e., dimethylnitrosamine (DMN), diethylnitrosamine (DEN), 4-aminophenol (4-APOL), 4-aminobiphenyl (4-ABPYL) and one direct unstable mutagen β-propiolactone (BPL). Under the conditions of the study, UMDH was concluded to be positive in the micronucleus assay using male mice at doses up to 56 mg/kg bw.

Covalent binding of UDMH and NDMA to the DNA of liver cells was shown by Sagelsdorff *et al*. (1988), where covalent binding index (CBI) values of 0.55, 26 and 2700 were calculated for daminozide, UDMH and NDMA, respectively. CBI is a measure of DNA damage due to covalent binding. Compounds with CBI of (i) > 1000 are regarded as potent carcinogens; (ii) of the order of 100 as moderately strong carcinogens; (iii) <10 weakly genotoxic carcinogens. Substances with CBI<1 are considered unlikely to induce tumours via DNA binding. Accordingly, a weak to moderate genotoxic potential can be concluded for UDMH, whereas NDMA can be regarded as rather potent carcinogen.

Despite the lack of reliable *in vitro* chromosome aberration assay, RAC concludes that there is no solid evidence for genotoxicity of UDMH *in vitro*. However, data from the open literature show that the compound is positive in an *in vivo* liver micronucleus assay and can bind covalently to liver DNA of rats treated orally with UDMH. Thus, a genotoxic potential for UDMH *in vivo* cannot be excluded.

<u>90-day oral toxicity studies</u>

90-day toxicity study in rats as well as mice is available. In rats (Anonymous, 1987a), no treatment-related toxic effects were observed up to dose levels of 125 ppm (8.98 mg/kg bw/day). In mice (Anonymous, 1987b), liver hypertrophy, karyomegaly, and accentuation of lobulation was observed in males of all treated groups at doses of ≥10 ppm (≥2 mg/kg bw/day). Alveolar/bronchial adenomas were observed at 100 ppm (20 mg/kg bw/day) in females and 250 ppm (50 mg/kg bw/day) in males. Thus, results of this study indicate that UDMH decreases the latency period for development of lung tumours found in the 2-year chronic carcinogenicity study (discussed in the section below).

<u>Carcinogenicity studies</u>

Chronic carcinogenicity studies with UDMH are available in rats and mice.

Fischer 344 rats (70/dose/sex) were exposed to UDMH (purity 86.4%) via drinking water at concentrations of 0, 1, 50 or 100 ppm (Anonymous, 1989a). The mean ingested doses over the two year period were 0, 0.07/0.09, 3.2/4.5, and 6.2/7.9 mg/kg bw/day for males/females, respectively. Satellite groups of 20 animals/sex/dose group were used for interim sacrifice at 12 months of treatment.

No treatment-related effects on survivor, clinical signs, and haematology were observed during the study. Differences between treated and control groups were noted in mean body weight (range ca. 2-5%) and food/water consumption values during specific study periods. No treatment-related macroscopic or microscopic lesions were reported at the interim sacrifice. An increased incidence of hepatocellular neoplasms (adenomas and carcinomas) was observed in females at all dose levels (0.1 - 8 mg/kg bw/day, Table below), and chronic inflammation of the liver was reported in all dose groups. The increase in the combined incidence of hepatocellular adenomas and carcinomas was not statistically significant in Fischer's exact test (p=0.029), however a significant dose-related trend was determined by

the Cochran Armitage trend analysis (p=0.01). Notably, the spontaneous incidence of hepatocellular neoplasms in female Fischer rats reported from the performing laboratory is very low (2/370 for adenoma, no carcinoma; 0.5%). The incidence rates in female rats is also higher than in published NTP historical controls (average 2.7%; range 0-10%).

With respect to dose selection, it is questionable if MTD was reached in this study. In the 90-day study, body weight gains in females were slightly reduced at 125 ppm (9 mg/kg bw/day), however the difference with the controls was not statistically significant (Johnson 1987a).

***Table**: Incidences of hepatocellular neoplasms in rats (terminal sacrifice) and chronic inflammation of the liver (12 months to termination) (Anonymous, 1989a)*

	Males				**Females**			
Dose (ppm)	**0**	**1**	**50**	**100**	**0**	**1**	**50**	**100**
Dose (mg/kg bw/d)	0	0.07	3.2	6.2	0	0.09	4.5	7.9
Hepatocellular adenoma	2/50	0/49	1/50	2/50	0/48	1/50	2/50	1/50
Hepatocellular carcinoma	1/50	0/49	0/50	1/50	0/48	0/50	3/50	4/50
Adenoma/carcinoma combined	3/50	0/49	1/50	3/50	0/48	1/50	5/50	5/50
Chronic inflammation of liver	1/50	5/49	6/50	13/50	12/48	13/50	22/50	22/50

Pituitary adenomas were reported for both male and female rats, but were more common in female rats. While pituitary adenomas are considered common spontaneous lesion in female Fischer rats (see HCD in previous section), it is noted that incidences exceeding the HCD for this tumour type were observed in carcinogenicity study with daminozide. The study concluded that UDMH did not reveal any carcinogenic potential.

Two long-term toxicity studies with UDMH in mice are available. In the first "low dose" study (Anonymous, 1989b), UDMH (purity not stated) was administered in drinking water to CD1 mice (90/dose/sex) at doses of 0, 1, 5 or 10/20 (m/f) ppm (doses ranging from approximately 0.2-2.7 mg/kg bw/day). Interim sacrifice was performed after 8 and 12 months of the study. Survival was significantly reduced in males at the top dose. The study followed OECD TG 451 and reported significantly increased incidences of pulmonary neoplasms (alveolar/bronchiolar adenoma and carcinoma) in the high dose females. The incidence of brown pigment in the liver was increased at ≥5 ppm (ca. 1.4 mg/kg bw/day), however the type of the pigment was not determined.

***Table**: Incidences of neoplastic lesions in the lung at the end of the study (n=50/sex/group)*

	Males				**Females**			
Dose (ppm)	**0**	**1**	**5**	**10**	**0**	**1**	**5**	**20**
Alveolar/bronchiolar adenoma	12/47	13/46	17/43	12/46	5/49	9/47	12/48	20/49
Alveolar/bronchiolar carcinoma	4/47	4/46	7/43	4/46	1/49	1/47	1/48	7/49
Adenoma/carcinoma combined	16/47	17/46	24/43	16/46	6/49	10/47	13/48	27/49

A second "high dose" UDMH study was subsequently conducted in CD1 mice (90/sex/dose group) at two dose levels of 0, 40 or 80 ppm in drinking water corresponding to 0, 7.3 or 21.8 mg/kg bw/day, respectively (Anonymous, 1990). Interim sacrifice was performed after 8 and 12 months of the study. The study is GLP compliant, however deviates from OECD TG 451

because only two dose groups were used instead of three. A significant increase in the incidence of neoplastic lesions in the liver (haemagiosarcoma) and lung (alveolar/bronchiolar adenoma/carcinoma) was observed in both treated groups. Mortality was severely increased at the top dose (only 1/50 males and 4/50 females survived until terminal sacrifice), and signs of significant hepatotoxicity such as accentuated liver lobulation, liver cell hyperthrophy and necrosis, presence of chronic inflammation and brown pigment, elevated levels of alanine aminotransferase and sorbitol dehydrogenase was reported from the study. Excessive mortality and clear liver toxicity indicate that the dosing probably exceeded the MTD.

***Table**: Absolute figures for survival and neoplastic lesions at the end of the study: n=50/sex/group (Anonymous, 1990)*

	Males			**Females**		
Dose (ppm)	**0**	**40**	**80**	**0**	**40**	**80**
Survival at the end of the study	15/50	12/50	1/50	21/50	4/50	4/50
Lung						
alveolar/bronchiolar adenoma	16/45	24/45	16/37	13/45	19/43	19/39
alveolar/bronchiolar carcinoma	3/45	9/45	3/37	1/45	4/43	3/39
adenoma/carcinoma combined	3/45	33/45	19/37	14/45	23/43	22/39
Liver						
hemangioma	0/45	1/45	0/37	1/45	1/43	0/39
hepatocellular adenoma	6/45	6/45	8/37	2/45	6/43	1/39
hemangiosarcoma	4/45	29/45	30/37	1/45	10/43	32/39
hepatocellular carcinoma	3/45	9/45	0/37	-	-	-

Overall conclusion regarding UDMH

Based on the reviewed data, genotoxicity of UDMH cannot be completely excluded. *In vitro* tests were mostly negative or inconclusive, however study protocols did not follow current guidelines and the purity of the substance was not always clearly stated. Open literature studies with UDMH showed its potential to induce micronuclei *in vivo* and to bind covalently to the liver DNA. However, these studies do not provide a definitive support for *in vivo* genotoxicity since the protection against oxidation of UDMH was not specified and mutagenic NDMA or other degradation products can be formed on the open air. The carcinogenicity study in rats was rather inconclusive, however a possible dose related trend in the increased combined incidence of hepatocellular adenomas and carcinomas in females can be noted. In addition, there is some concern that the top dose of 6-8 mg/kg bw/day did not reach the MTD since only decreased body weight and food/water consumption, and increased incidences of cloudy corneas and corneal mineralization were reported from the study. The "high dose" mouse study suggests a possible association between exposure to UDMH and the occurrence of neoplastic lesions in the lung (alveolar/brionchiolar adenomas and carcinomas) and malignant liver tumours (hemangiosarcomas) from 40 ppm (7.3 mg/kg bw/day), a dose for which excessive systemic toxicity (mortality) was not observed. In the "low dose" mouse study, the incidence of alveolar/bronchiolar adenomas and carcinomas was significantly increased in the top dose females (ca. 3 mg/kg bw/day).

In a recent renewal application to EFSA, EU Daminozide Task Force provided some mechanistic consideration regarding the toxicity assessment of UDMH. In a study by Cabral *et al*. (1995), carcinogenicity of daminozide alone and in combination with UDMH was

investigated in a medium-term initiation-promotion liver bioassay with Fischer 344 rats (Ito model). Treatment with daminozide alone (20000 ppm), UDMH alone (75, 150, 300 ppm), or UDMH in combination with daminozide did not induce an increase in the number and/or size of GST-P positive foci in the liver as an established precursor of liver carcinogenicity. RAC shares the view of the The Panel on Plant Protection Products and their Residues (PPR Panel) and DS that while the Ito model has a high positive predictive value for liver carcinogenicity in rats and mice, its ability to identify reliably non-carcinogens (i.e., negative predictivity) is rather uncertain. Therefore, the study cannot be used to disregard the liver carcinogenicity observed in the chronic studies with UDMH.

With respect to lung carcinogenicity, the high spontaneous lung tumour incidence in CD-1 mice may raise some reservations on the validity of the above results. Further, Hastings *et al.* (1989) showed that lung tumour incidence might increase considerably when instead of one lung section multiple sections are evaluated. As pointed out by the Rapporteur Member State (RMS), histopathology in both mice studies was properly done, with equal treatment of samples from control and treated animals. Thus, the tumour findings in these studies are a clear result that should be considered during evaluation of UDMH.

Considering the overall weight of evidence, RAC concludes that UDMH has a carcinogenic potential for which a genotoxic mode of action cannot be completely excluded. In a previous assessment (The EFSA Journal, 61, 1-27), PPR concluded that UDMH is not a genotoxic carcinogen and threshold doses of UDMH can be used, though with caution. Other agencies such as IARC and the EPA consider UDMH as a Group 1B carcinogen. The current Annex VI entry of UDMH as Carc. 1B is more than 30 years old and the basis of classification is not available.

2.6.6 Summary of reproductive toxicity [equivalent to section 10.10 of the CLH report template]

2.6.6.1 Adverse effects on sexual function and fertility – generational studies

Table 45: Summary table of animal studies on adverse effects on sexual function and fertility – generational studies

Method, guideline, deviations[1] if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results** **- NOAEL/LOAEL (for sexual function and fertility, parents)** **- target tissue/organ** **- critical effects at the LOAEL**	**Reference**
Two-generation reproduction toxicity study OECD TG 416 Deviations: The dose interval should not exceed 3 fold. The fourth group should be involved. Sperm parameters and primordial follicles were not evaluated Organs were not weighed at the study termination Rat (Sprague-Dawley) male/female 30 animals/group (F0) 25 animals/group (F1) **Acceptable study**	Test material: daminozide Purity: > 99% Form: powder Oral: gavage Dose levels: 0, 60, 360 or 1200 mg/kg bw/day Duration of exposure: F0: for ten weeks, then throughout mating, gestation, lactation, until sacrifice; F1: in utero, while nursing, then from Day 25 post-partum throughout mating, gestation, lactation, until sacrifice	NOAEL (parental toxicity): 360 mg/kg bw/day LOAEL(parental toxicity): 1200 mg/kg bw/day Critical effects: clinical signs (loose faeces from Week 4 in F0 as well as F1 animals; perianal fur staining in all F0 animals from Week 10 and F1 animals from Week 6; excessive post-dose salivation in all F0 as well as F1 animals from Week 11; these effects were not observed at lower doses) NOAEL (reproductive toxicity): 1200 mg/kg bw/day (top dose)	*Anonymous (1994)*
Two-generation reproduction toxicity study OECD TG 416	Test material: Alar Purity: 99% Oral route: in diet Dose levels: 0, 100,	NOAEL (parental toxicity): 50 mg/kg bw/day (1000 ppm) LOAEL (parental toxicity): 500 mg/kg bw/day (10000 ppm)	*Anonymous (1987*)

Method, guideline, deviations[1] if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results** **- NOAEL/LOAEL (for sexual function and fertility, parents)** **- target tissue/organ** **- critical effects at the LOAEL**	**Reference**
Deviations: The dose interval should not exceed 3 fold. The fourth group should be involved. Sperm parameters and primordial follicles were not evaluated Organs were not weighed Rat (albino Crl:CD (SD)BR) male/female 25 animals/group **Acceptable study**	1000 and 10000 ppm Duration of exposure: F0: continuously from approximately 7 weeks of age throughout mating, gestation, lactation, until sacrifice; F1: in utero, while nursing; continuously in the diet after weaning throughout mating, gestation, lactation, until sacrifice	Critical effects: changes in body weight in F1 males (significant decrease by 8 – 9% at Week 15 – 19; *see Table 2.6.6.1.1-2*) NOAEL (reproductive toxicity): 500 mg/kg bw/day (10000 ppm, the top dose)	

Table 46: Summary table of human data on adverse effects on sexual function and fertility

Type of data/report	**Test substance**	**Relevant information about the study (as applicable)**	**Observations**	**Reference**	**Type of data/report**
No data available					

Table 47: Summary table of other studies relevant for toxicity on sexual function and fertility

Type of data/report	**Test substance**	**Relevant information about the study (as applicable)**	**Observations**	**Reference**	**Type of data/report**
No data available					

2.6.6.1.1 Short summary and overall relevance of the provided information on adverse effects on sexual function and fertility – generational studies

Two generational studies were performed in compliance with OECD TG 416. In the dietary two-generation reproduction study in rats *(Anonymous, 1987)*, the significant decrease in body weight in F1 males of the top dose group was observed *(see Table 2.6.6.1.1-2)*. In parental animals, the effect on body weight and body weight gain was not revealed *(see Table 2.6.6.1.1-1).* No treatment-related clinical observations or mortality were noted in adult animals or pups during the study. No treatment-related effects were found in F0 or F1 animals at gross necropsy and histopathological examination. There were no differences in reproductive function (fertility, length of gestation, litter size, sex ratio of pups, etc.; *see Table 2.6.6.1.1-4 and Table 2.6.6.1.1-5)* comparing treated F0 or F1 animals to controls. Based on the results of this study, the NOAEL for parental toxicity was set at 50 mg/kg bw/day, whereas the NOAEL for reproduction and developmental effects at 500 mg/kg bw/day (top dose).

Table 2.6.6.1.1-1: Body weights (g) in F0 animals; *(Anonymous, 1987)*

Dose (ppm) **Week**	**0**	**100**	**1000**	**10000**
Males				
0	263.2	265.3	264.3	264.7
1	311.5	315.4	316.2	316.8
2	349.9	353.7	356.7	354.4
3	385.4	388.9	391.6	388.4
4	416.0	419.2	424.2	420.4
5	432.3	436.3	443.4	437.0
6	455.0	460.5	468.3	458.2
7	474.3	479.8	487.5	475.5
8	493.1	498.3	506.0	491.3
9	512.6	516.1	524.2	506.5
10	524.3	530.6	537.5	520.7
11	528.3	536.3	542.7	521.8
12	540.5	546.0	552.5	536.9
13	546.0	550.4	560.2	540.6
14	554.2	563.2	571.2	548.1

15	565.0	571.8	578.6	567.2
Females				
0	164.4	160.4	164.3	167.1
1	184.6	181.2	185.6	190.0
2	199.5	194.7	201.2	204.7
3	213.3	205.6	213.6	218.0
4	226.0	219.2	226.9	229.5
5	232.0	224.4	232.0	235.4
6	241.0	233.7	241.7	244.3
7	247.2	236.4	246.9	251.4
8	253.4	246.3	251.8	257.9
9	261.4	252.5	259.6	264.2
10	265.5	254.4	261.1	265.9

Table 2.6.6.1.1-2: Body weights (g) in F1 males; * = $p \leq 0.5$

Dose (ppm) **Week**	**0**	**100**	**1000**	**10000**
0	113.7	113.3	118.9	108.8
1	174.9	173.6	182.2	168.6
2	233.5	231.2	239.6	223.9
3	295.6	291.0	298.4	281.6
4	340.8	334.3	341.9	327.1
5	380.0	370.6	380.1	362.2
6	414.4	399.7	413.2	393.4
7	440.3	424.4	437.8	419.2
8	459.0	443.5	455.5	439.0
9	486.0	467.6	480.8	462.0
10	506.4	483.2	497.0	474.3

11	524.1	494.7	513.0	486.1*
12	528.0	503.2	519.1	491.4*
13	539.3	517.0	532.2	502.9
14	555.1	531.3	543.0	513.2
15	564.4	538.5	549.9	521.2*
16	579.8	554.2	563.1	534.4*
17	588.8	563.5	574.1	539.7*
18	602.6	573.0	584.1	550.2*
19	607.8	579.0	588.4	557.2*

Table 2.6.6.1.1-3: Body weights (g) in F1 females; * = $p \leq 0.5$

Dose (ppm) **Week**	**0**	**100**	**1000**	**10000**
0	99.9	98.2	105.0	97.8
1	138.8	137.8	141.9	132.9
2	166.7	163.3	168.4	163.8
3	192.7	186.7	192.0	188.9
4	210.5	202.5	208.6	208.2
5	227.1	216.5	224.9	224.6
6	240.9	229.5	237.9	237.9
7	250.4	239.5	250.1	248.4
8	256.2	244.9	255.4	252.8
9	269.3	257.9	266.6	267.3
10	275.2	264.0	272.6	271.3
11	279.0	267.7	275.9	276.0
12	300.7	282.1	294.2	290.7
Lactation (days post-partum)				
0	309	292	311	299

4	306	289	308	306
7	314	302	316	315
14	331	313*	329.3	327.0
21	316	301*	318	313

Table 2.6.6.1.1-4: Mean pup weights (g); * = $p \leq 0.5$

Dose levels	**day 1**	**day 4**	**day 7**	**day 14**	**day 21**
F_1 0 ppm	6.4	10.3	16.7	32.1	51.5
100 ppm	6.3	10.1	16.7	32.4	51.3
1000 ppm	6.2	10.3	17.0	33.2	52.4
10000 ppm	6.4	10.1	16.2	31.6	49.1
F_2 0 ppm	6.4	10.7	17.6	33.7	54.2
100 ppm	6.3	10.2	16.4*	32.3	52.9
1000 ppm	6.5	11.0	17.8	34.3	55.9
10000 ppm	6.5	11.0	17.6	33.19	54.1

Table 2.6.6.1.1-5: Group mating data and group mean litter data in F0 and F1 generation

Parameter \ Dose[ppm]	F0 generation				F1 generation			
	0	**100**	**1000**	**10000**	**0**	**100**	**1000**	**10000**
Fertility index in males [%]	100	92	96	92	92	96	100	92
Fertility index in females [%]	100	100	100	100	92	96	100	100
Mating [%]	100	92	96	92	92	96	100	92
Gestation [%]	100	100	100	100	100	100	100	100
Viability	97	98	96	99	98	97	99	98
Weaning [%]	100	100	99	99	99	100	99	100
No. of pups born alive/No. of pups born dead	316/0	305/1	309/4	309/4	297/5	310/6	329/0	297/6
No. of pups born (Mean no. / female)	316 (12.2)	306 (13.3)	313 (13.0)	313 (13.6)	302 (13.1)	316 (13.2)	329 (13.2)	303 (13.2)

In the second, gavage two-generation reproduction study in rats *(Anonymous, 1994)*, two control F0 females were sacrificed prematurely. One showed red fluid discharge from the mouth during dosing and the other dystocia and incomplete parturition. At 60 mg/kg bw/day, one female was found dead in the first week of dosing. In the F1 generation, one male treated at 60 mg/kg bw/day died following a dosing intubation error and one female from the same group was sacrificed prematurely during lactation because of a mammary mass. None of these was considered to be related to the test material treatment. There were no effects of treatment at any dose level on parental bodyweight, food consumption, mating and fertility or on pup survival, weight and clinical

condition *(see Table 2.6.6.1.1-7)*. No treatment-related abnormalities were observed at gross necropsy of the parental animals or offspring as well as at histopathological examination of organs/tissues of the parental animals. In the 1200 mg/kg bw/day group, clinical signs in parental animals were noted in both generations. This included loose and/or odorous faeces (from Week 4), perianal fur staining (from Week 6 or 10) and post-dose salivation (from Week 11). There was also evidence of increased water consumption but this was only measured and confirmed for F0 generation males *(see Table 2.6.6.1.1-6)*. In the 360 and 60 mg/kg bw/day groups, no treatment-related effects were noted. The NOAEL for the parental toxicity was set at 360 mg/kg bw/day based on clinical signs described above, which were observed in both F0 and F1 animals of the top dose group. The NOAEL for the reproductive and developmental toxicity was established at 1200 mg/kg bw/day (top dose) as no treatment-related adverse effects were observed.

Table 2.6.6.1.1-6: Group mean water consumption (g/rat/day) in F0 males; ** = $p \leq 0.1$

Group / Days	1 (control)	2 (60 mg/kg bw/day)	3 (360 mg/kg bw/day)	4 (1200 mg/kg bw/day)
86-91	51.5	46.9	50.7	658.4**

Table 2.6.6.1.1-7: Group mean pregnancy and litter data

Dose [mg/kg bw/day] / Parameter	F0 generation				F1 generation			
	0	**60**	**360**	**1200**	**0**	**60**	**360**	**1200**
Fertility index in males [%]	92.6	96.6	96.6	100.0	95.8	81.8	100.0	96.0
Fertility index in females [%]	93.1	96.6	96.7	100.0	95.8	80.0	96.0	96.0
Gestation index	100	100	100	100	100	100	100	100
Mean no. of pups born	13.7	13.8	14.2	13.6	14.1	13.5	13.2	14.0
Mean live birth index	99.2	98.5	97.1	98.4	97.3	98.9	99.2	95.0
Mean viability index	95.7	98.8	96.4	96.6	92.7	98.6	98.3	97.2
Mean lactation index	100.0	97.8	99.1	99.6	99.4	98.7	99.5	98.9
Sex ratio at birth	48:52	50:50	53:47	53:47	55:45	56:44	49:51	47:53

2.6.6.1.2 Comparison with the CLP criteria regarding adverse effects on sexual function and fertility

According to CLP (Regulation (EC) No. 1272/2008), an active substance meets the criteria for classification in relation to sexual function and fertility, if it induces alterations to the female and male reproductive system, adverse effects on onset of puberty, gamete production and transport, reproductive cycle normality, sexual behaviour, fertility, parturition, pregnancy outcomes, premature reproductive senescence, or modifications in other functions that are dependent on the integrity of the reproductive systems. In two-generation reproduction studies with daminozide, no treatment-related adverse effects on sexual function and fertility were observed.

2.6.6.2 Adverse effects on development [equivalent to section 10.10.4 of the CLH report template]

Table 48: Summary table of animal studies on adverse effects on development

Method, guideline, deviations1 if any, species, strain, sex, no/group	**Test substance, dose levels, duration of exposure**	**Results** **- NOAEL/LOAEL (for parent, offspring and for developmental effects)** **- target tissue/organ** **- critical effects at the LOAEL**	**Reference**
Prenatal development toxicity study OECD TG 414 Rat (Sprague-Dawley) 25 females/group Acceptable study	Test material: daminozide Oral route: by gavage Dose levels: 0 (vehicle control: water), 150, 750 and 1500 mg/kg bw/day Duration of exposure: once daily between Days 6 and 15 of pregnancy Purity: >99% Form: crystalline	NOAEL (maternal toxicity): 150 mg/kg bw/day based on significantly ($p < 0.01$ – $p< 0.001$) reduced body weight gain at 750 (by 30.8%) and 1500 mg/kg bw/day (by 35.5%) during Weeks 6 – 9 (see Table 2.6.6.2.1-1) NOAEL (developmental toxicity): 1500 mg/kg bw/day No teratogenic effects were observed	*Anonymous (1993)*
Prenatal development toxicity study OECD TG 414 Rabbit (New Zealand White) 5 females/group Supplementary study performed to set doses for the definite study	Test material: daminozide oral: by gavage Dose levels: 300, 500 and 700 mg/kg bw/day; 1000 mg/kg bw/day for 14 days (study extension) Vehicle: 0.5% w/v aqueous methylcellulose Exposure: days 7 to 28 of gestation Purity: 100% Form: powder	1000 mg/kg bw/ day was the maximum tolerated dose. Doses of 250, 500 and 1000 mg/kg bw/day were proposed to be used in the definitive study	*Anonymous (2006a)*

Prenatal development toxicity study OECD TG 414 Deviations: At the end of the study only 15 and 8 pregnant females were alive in the 500 and 1000 mg/kg bw/day group, respectively. However, each test group should contain approximately 20 pregnant females at necropsy, groups with fewer than 16 animals may be inappropriate. Maternal mortality should not exceed 10 percent, which was not met in the study. Rabbit (New Zealand White) 25 females/group Acceptable study	Test material: daminozide Oral: by gavage Purity: 99.5% Form: powder Dose levels: 0, 250, 500 and 1000 mg/kg bw/day Vehicle: carboxymethyl cellulose (0.5% w/v) Exposure: days 6 to 28 of presumed gestation	NOAEL (maternal toxicity): 250 mg/kg bw/day LOAEL (maternal toxicity): 500 mg/kg bw/day Adverse effects at LOAEL: (see Table 2.6.6.2.1-6): mortality (36% vs. 4% in control; $p < 0.05$) and adverse clinical observations (soft/liquid faeces: 80% vs. 36% in control; $p < 0.05$; hyperpnoea: 16% vs. 0%; $p < 0.01$; hyperactivity: 12% vs. 0% in control; $p < 0.05$; convulsions: 12% vs. 0% in control; non-significant) NOAEL (developmental toxicity): 500 mg/kg bw/day LOAEL: (developmental toxicity): 1000 mg/kg bw/day Adverse effects: slight reduction in ossification (see Section 2.6.6.2.1 and Tables 2.6.6.2.1-10 and 2.6.6.2.1-11) and foetal weight (non-significantly ↓ by 10%; bw in male foetuses per litter ↓ by 15.3%; $p < 0.05$; see Table 2.6.6.2.1-9) No teratogenic effects were observed	*Anonymous (2006b)*

Prenatal development toxicity study OECD TG 414 Deviations: More animals should have been enrolled in the study. At the end of the study only 12, 14, 15, and 8 pregnant females were alive in the 0, 50, 150, and 300 mg/kg group, respectively. At necropsy, the groups consisting of approximately 20 females with implantation sites are recommended. Gravid uteri including the cervix were not weighed Rabbit (Dutch Belted) 16 females/group Acceptable study	Test material: Alar technical Oral: by gavage Purity: daminozide forms 99 % w/w (1% innert ingredients) Form: granules Dose levels: 50, 150, and 300 mg/kg bw/day Vehicle: carboxymethylcellulose (0.5%) Exposure: once daily on days 7 - 19 of gestation	NOAEL (maternal toxicity and developmental toxicity): 300 mg/kg bw/day No teratogenic effects were observed	*Anonymous (1985)*
Teratogenicity study No TG, GLP Supplementary study (literature data) Rat 20 females/group	Test material: daminozide Oral: by gavage Dose levels: 0, 300, 600, and 1000 mg/kg bw/day Exposure: Day 6 - 15 Vehicle: distilled water Purity: >99%	NOAEL (maternal and developmental toxicity): 1000 mg/kg bw/day	Khera (1979) Report Environ J., Sci. Health; 1979 Vol. B14 (6): 563-577

Table 49: Summary table of human data on adverse effects on development

Type of data/report	**Test substance**	**Relevant information about the study (as applicable)**	**Observations**	**Reference**	**Type of data/report**
No data available					

Table 50: Summary table of other studies relevant for developmental toxicity

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference	Type of data/report
No data available					

2.6.6.2.1 Short summary and overall relevance of the provided information on adverse effects on development

One acceptable teratogenicity study in rats and two in rabbits are available. In rats *(Anonymous, 1993)*, there were no unscheduled mortalities amongst treated animals and no treatment-related changes in clinical condition or abnormalities at necropsy. One control female was found dead on Day 16 of pregnancy. Necropsy revealed uterine haemorrhage. There was a dose-related effect of treatment at 1500 mg/kg bw/day and to a lesser extent, at 750 mg/kg bw/day on bodyweight gain and food consumption *(see Table 2.6.6.2.1-1 and Table 2.6.6.2.1-2)* . There were no adverse effects of treatment at any dose level on numbers of implantations and live foetuses or on post-implantation losses. The sex distribution of the live foetuses was similar in all groups (*see Table 2.6.6.2.1-3: Pregnancy and foetal data)*. Mean foetal weights were marginally lower (not statistically significantly) in the groups treated at 750 and 1500 mg/kg bw/day than in the control group (by 3%; *see Table 2.6.6.2.1-3*). This was probably related to the slightly larger live litter size (by 10.7% comparing to control) and/or maternal toxicity characterised by reductions in food consumption as well as retardations of bodyweight gain and was not regarded as treatment-related. Mean foetal weights at 150 mg/kg bw/day were marginally greater than in the control group. There were no adverse effects of treatment at any dose level on the nature or incidence of major or minor external, visceral or skeletal abnormalities or on the incidences of foetuses with variants of development. The maternal NOAEL was established at 150 mg/kg bw/day based on the reduced body weight gain in 750 and 1500 mg/kg bw/day dose groups. Since the decreased foetal body weight was not significant (by 3%) andconsidered to be attributed to the larger litter size and reduced maternal body weight gain, the developmental and teratogenicity NOAEL were established at 1500 mg/kg bw/day (top dose). This conclusion is in accordance with CLP Regulation(EC) No. 1272/2008 saying that: “Based on pragmatic observation, maternal toxicity may, depending on severity, influence development via non-specific secondary mechanisms, producing effects such as depressed foetal weight.” The corrected maternal body weight was reduced in 750 and 1500 mg/kg bw/day dose groups only marginally (approximately by 1.5% at the top dose comparying to the control), (see Table 2.6.6.2.1-1).

Table 2.6.6.2.1-1: Maternal body weights gain (g); gravide uterine weight (g) and corrected maternal body weight (g); corrected maternal body weight = body weight on day 20 of gestation minus the gravid uterine weight; *(Anonymous, 1993)*

Dose / **Days of pregnancy**	**0 (mg/kg bw/day)**	**150 (mg/kg bw/day)**	**750 (mg/kg bw/day)**	**1500 (mg/kg bw/day)**
0 to 6	29.0	30.8	30.1	29.9
6 to 7	6.5	4.9	2.0***	2.1***
6 to 8	10.3	9.1	7.1**	5.8***
6 to 9	17.2	15.8	11.9***	11.1***
9 to 12	20.7	20.5	18.3	17.1
12 to 15	25.5	29.2	29.3	27.6
15 to 20	83.3	83.2	84.6	87.8
6 to 15	63.3	65.5	59.5	55.8*
Gravide uterine weight	99	101.6	101.1	102.6
Corrected maternal body weight	330	333.4	326.9	325.4
No. of animals in group	23	25	24	25

Statistically significant: * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$

Table 2.6.6.2.1-2: Food consumption (g/rat/day)

Dose / Days of pregnancy	0 (mg/kg bw/day)	150 (mg/kg bw/day)	750 (mg/kg bw/day)	1500 (mg/kg bw/day)
0 to 6	24.8	25.4	25.0	24.8
6 to 9	27.6	27.8	26.0	25.4*
9 to 12	29.5	30.2	28.2	27.5*
12 to 15	31.8	32.2	31.8	31.6
15 to 18	32.4	33.8	33.8	33.2
18 to 20	30.8	31.8	31.4	32.2
No. of animals in group	23	25	24	25

Statistically significant: * = $p < 0.05$;

Table 2.6.6.2.1-3: Pregnancy and foetal data

Dose / Parameter	0 (mg/kg bw/day)	150 (mg/kg bw/day)	750 (mg/kg bw/day)	1500 (mg/kg bw/day)
No. of pregnant females	24	25	25	25
No. of corpora lutea	16.5	17.3	17.6	17.9
No. of implantation	15.2	15.6	15.9	16.5
No. of live foetus	14.0	14.8	14.8	15.5
Pre-/Post-implantation loss (%)	9.1 / 11.3	9.4 / 4.5	8.7 / 7.5	6.9 / 6.3
Sex ratio	49 : 51	52 : 48	46 : 54	49 : 51
Foetal weight (g)	4.09	4.13	3.97	3.97

1 dam did not give birth to live foetuses, i.e. 23 litters in Group 1 and 24 litters in Group 3 were included in the statistics.

Table 2.6.6.2.1-4: Foetal examination data (Mean % = sum of % of affected foetuses per litter/number of litters)

Dose / Parameter	0 (mg/kg bw/day)	150 (mg/kg bw/day)	750 (mg/kg bw/day)	1500 (mg/kg bw/day)
External and visceral examination				
No. of foetuses (litters)	337 (23)	371 (25)	369 (24)	387 (25)
No. with minor abnormalities only/**Mean %**	1 (1) / **0.3**	3 (3) / **0.9**	4 (4) / **1.0**	5 (4) / **1.3**
No. with major abnormalities/**Mean %**	0 (0) / **0.0**	2 (2) / **0.6**	0 (0) / **0.0**	0 (0) / **0.0**
Skeletal examination				
No. of foetuses (litters)	169 (23)	185 (25)	185 (24)	194 (25)
No. with minor abnormalities only/**Mean %**	4 (4) / **2.4**	13 (10) / **7.4**	13 (11)* / **6.6**	7 (6) / **3.5**
No. with major abnormalities/**Mean %**	0 (0) / **0.0**	1 (1) / **0.7**	0 (0) / **0.0**	0 (0) / **0.0**
Combined examination				
No. with any major abnormalities/ **Mean, %**	0 (0) / **0.0**	2 (2) / **0.6**	0 (0) / **0.0**	0 (0) / **0.0**

Note: It could seem that the values in Table 2.6.6.2.1-3 do not correspond with values in the Table 2.6.6.2.1-4 because in the Table 2.6.6.2.1-3 all pregnant dams were included in the statistics (i.e. 24, 25, 25 and 25 at 0, 150, 250, 750 and 1500 mg/kg bw/day, resp.), whereas in the Table 2.6.6.2.1-4 "Foetal examination data" females without live foetuses were excluded (1 in control and 1 in Group 3, i.e. 23, 25, 24, and 25 litters at 0, 150, 250, 750 and 1500 mg/kg bw/day, resp.). According to individual pregnancy data in the original study report, the control group contained 337 pups. This number is the same for 23 as well as 24 litters because the dam No. 10 did not have live pups.

Table 2.6.6.2.1-5: Examination of foetuses; group mean data, ( ) = Mean [%]; **Mean % = sum of % of affected foetuses per litter/number of litters)**

Dose / **Findings**	**0 (mg/kg bw/day)**	**150 (mg/kg bw/day)**	**750 (mg/kg bw/day)**	**1500 (mg/kg bw/day)**
External and visceral examination				
Total number of foetuses (litters) examined	337 (23)	371 (25)	369 (24)	387 (25)
Cleft palate (**major**)	0 (0.0)	1 (0.3)	0 (0.0)	0 (0.0)
Palate secondary: undeveloped areas in midline (**variant**)	2 (0.6)	4 (1.1)	2 (0.5)	8 (2.0)
Innominate artery: absent. Right common carotid &right subclavian arteries arising directly from aortic arch (**minor**)	0 (0.0)	3 (0.9)	4 (1.0)	4 (1.0)
Umbilical hernia (**major**)	0 (0.0)	1 (0.3)	0 (0.0)	0 (0.0)
Abdominal haemorrhage (**minor**)	1 (0.3)	0 (0.0)	0 (0.0)	1 (0.3)
Kidneys: uni- or bilateral: increased pelvic cavitation (**variant**)	32 (10.1)	**13 (3.3)****	42 (11.0)	27 (7.0)
Ureter: uni- or bilateral: dilated (**variant**)	52 (15.4)	**27 (7.4)***	58 (15.1)	57 (14.8)
Skeletal examination: Skull				
Total number of foetuses (litters) examined	169 (23)	185 (25)	185 (24)	194 (25)
Cleft palate (**major**)	0 (0.0)	1 (0.7)	0 (0.0)	0 (0.0)

Hyoid: not ossified (**variant**)	2 (1.0)	1 (0.7)	5 (2.3)	4 (1.9)
Occipital: retarded ossification (**variant**)	1 (0.5)	5 (3.1)	5 (2.6)	3 (1.3)
Interparietal: retarded ossification (**variant**)	8 (4.7)	15 (8.6)	16 (8.2)	17 (8.7)
Parietals: retarded ossification (**variant**)	2 (1.1)	3 (1.9)	10 (4.8)	3 (1.6)
Temporals: retarded ossification (**variant**)	0 (0.0)	1 (0.7)	1 (0.5)	1 (0.7)

Statistically significant: * = $p < 0.05$; ** = $p < 0.01$;

According to the authors of the published paper *Khera (1979)*, no signs of toxicity were observed in treated rats, and pregnancy rates, numbers of corpora lutea, implantation and resorption rates, foetal deaths, sex ratio, foetal weights, number of live foetuses, the incidence of foetal anomalies and skeletal malformations were not significantly different from control values. The NOAELs for maternal as well as developmental toxicity is established at 1000 mg/kg bw/day. However, this study is considered to be supplementary for the overall evaluation since not all observations were reported and no individual data were presented.

In rabbits (*Anonymous, 2006b)*, administration of the test material at 500 and 1000 mg/kg bw/day resulted in the death of 7 and 8 animals and the early sacrifice of 2 and 6 animals, respectively. Each of these deaths (with the exception of one death in each of these groups that was considered to be the result of intubation accidents) was considered to be treatment-related because they were preceded by adverse clinical observations and/or reductions in body weight gain *(see Table 2.6.6.2.1-7)* and feed consumption *(see Table 2.6.6.2.1-8)*. In addition, two animals in the 1000 mg/kg bw/day group aborted and were sacrificed. These abortions were also considered to be test material related *(see Table 2.6.6.2.1-6)*. The number of animals with soft or liquid faeces, ungroomed coat, hyperactivity, perinasal or perioral substance, hyperpnoea, convulsions (clonic or tonic), tremors, impaired righting reflex, and gasping was increased or significantly increased in the 500 and 1000 mg/kg bw/day groups. These observations were considered to be test material related and generally occurred in the animals that did not survive to scheduled sacrifice. In the 1000 mg/kg bw/day group, the number of animals with scant faeces, mucoid faeces, decreased motor activity, dehydration, dyspnoea, ptosis, blue or light blue colouring around the mouth and cold to touch was significantly increased. Twitches and mydriasis also occurred in the 1000 mg/kg bw/day group *(see Table 2.6.6.2.1-6)*. All gross lesions in the 500 and 1000 mg/kg bw/day groups occurred in the animals that were found dead or sacrificed early. All were considered secondary to the clinical observations. Pregnancy occurred in 24 animals in each group. Caesarean-sectioning observations were based on 23, 23, 15, and 8 pregnant animals with one or more live foetuses in the 0, 250, 500 and 1000 mg/kg bw/day groups, respectively, which survived to DG 29. Foetal weights were reduced by 10% in the 1000 mg/kg bw/day group (bw in male foetuses/litter was significantly decreased by 15.3%) when compared to controls *(see*

Table 2.6.6.2.1-9). The number of foetuses with alterations was significantly increased in the 1000 mg/kg bw/day group. This included a significant increase in the number of foetuses with thickened ribs *(see Table 2.6.6.2.1-10)* and decrease in the average number of ossified forelimb phalanges *(see Table 2.6.6.2.1-11).* No other gross external, soft tissue or skeletal foetal alterations (malformations or variations) or differences in ossification sites per litter were caused by the test material. Under the conditions of the study, the maternal NOAEL was set at 250 mg/kg bw/day (the 500 and 1000 mg/kg bw/day doses caused adverse clinical observations and mortality). The developmental NOAEL is supported to be 500 mg/kg bw/day based on higher incidence of abortions, slight reduction in ossification and foetal weight occurring at 1000 mg/kg bw/day. It should be, however, mentioned that 1000 mg/kg bw/day is recommended as a limit dose according to OECD TG 414; and decreased foetal weight as well as reduced ossification were observed at this dose in the presence of maternal toxicity (excessive mortality, clinical observations described above, decreased food consumption). The changes in the corrected maternal body weights were not found (see Table 2.6.6.2.1-7). The CLP Regulation (EC) No. 1272/2008) says that a) "Classification is not necessarily the outcome in the case of minor developmental changes, when there is only a small reduction in foetal/pup body weight or retardation of ossification when seen in association with maternal toxicity"; b) "Adverse effects on reproduction seen only at very high dose levels in animal studies (for example doses that induce prostration, severe inappetence, excessive mortality) would not normally lead to classification". No treatment-related teratogenic effects were observed.

Table 2.6.6.2.1-6: Clinical observation; (*Anonymous, 2006b)*

Dose / **Findings**	**0 (mg/kg bw/day)**	**250 (mg/kg bw/day)**	**500 (mg/kg bw/day)**	**1000 (mg/kg bw/day)**
Number of females	25	25	25	25
Mortality	1	1	**9***	**14****
Found dead	0	0	**7****	**8****
Moribund sacrificed	1	1	2	6
Aborted and sacrificed	0	0	0	2
Soft and liquid faeces	9	9	**20***	**25****
Ungroomed coat	4	5	14	**24****
Scant faeces	3	5	5	**16****
Mucoid faeces	0	1	2	**12****
Decreased motor activity	0	0	1	**7****
Hyperactivity	0	0	**3***	**5****
Dehydration	1	0	0	**6****
Dyspnoea	0	0	1	**4****

Ptosis	0	0	0	**4****
Perinasal substance	0	0	**4****	**3****
Hyperpnoea	0	0	**4****	**3****
Convulsion	0	0	3	3
Tremors	0	0	1	3
Blue mouth	0	0	0	**3****
Cold to touch	0	0	0	**3****
Perioral substance	0	0	1	2
Red substance in cage pan	1	2	0	2
Twitches	0	0	0	2
Mydriasis	0	0	0	2
Impaired righting reflex	0	0	2	1
Gasping	0	0	1	1

Statistically significant: * = $p < 0.05$; ** = $p < 0.01$;

Table 2.6.6.2.1-7: Maternal body weight changes (kg); gravide uterine weight (g) and corrected maternal body weight (kg); statistically significant: * = p < 0.05; ** = p < 0.01; corrected maternal body weight = body weight on day 29 of gestation minus the gravid uterine weight; (*Anonymous, 2006b)*

Dose / Days	0 (mg/kg/day)	250 (mg/kg/day)	500 (mg/kg/day)	1000 (mg/kg/day)
0 to 6	0.13	0.16	0.15	0.09
6 to 9	0.00	0.03	0.03	-0.06
9 to 12	0.03	0.02	-0.02	-0.06
12 to 15	0.10	0.06	0.03	0.01
15 to 19	0.07	0.06	0.08	-0.02*
19 to 24	0.10	0.11	0.09	-0.00**
24 to 29	0.00	0.10*	0.11*	0.09
6 to 29	0.34	0.44	0.44	0.27
0 to 29	0.47	0.61*	0.61*	0.41
Gravid uterine weight	509.2	519.6	503.3	484.1
Corrected maternal body weight	3.31	3.43	3.40	3.35

Table 2.6.6.2.1-8: Maternal feed consumption (g/kg/day); Statistically significant: * = p < 0.05; ** = p < 0.01; (*Anonymous, 2006b)*

Dose / Days	0 (mg/kg/day)	250 (mg/kg/day)	500 (mg/kg/day)	1000 (mg/kg/day)
6 to 9	45.4	45.4	43.2	30.0**
9 to 12	42.4	41.4	40.7	23.3**
12 to15	42.4	42.0	40.6	24.2**
15 to 19	46.2	42.7	45.7	29.9**
19 to 24	41.3	39.4	44.0	32.4*
24 to 29	23.8	32.9*	36.3**	30.8
6 to 29	39.4	40.6	42.1	34.7

Table 2.6.6.2.1-9: Caesarean-sectioning and litter observations

Dose / **Findings**	**0 (mg/kg bw/day)**	**250 (mg/kg bw/day)**	**500 (mg/kg bw/day)**	**1000 (mg/kg bw/day)**
Pregnant	24 (96 %)	24 (96%)	24 (96%)	24 (96%)
Found dead	0	0	**7 (29%)****	**8 (33%)****
Moribund sacrificed	1 (4%)	1 (4%)	2 (8%)	6 (25%)
Aborted and sacrificed	0	0	0	2 (8%)
No. of caesarean-sectioned animal	23	23	15	8
Corpora lutea	9.0 ± 1.7	9.0 ± 2.1	8.1 ± 1.6	9.6 ± 2.7
Live foetuses	195	194	119	72
Implantation	8.8 ± 1.5	8.7 ± 2.4	8.1 ± 1.6	9.2 ± 3.0
Litter sizes	8.5 ± 1.6	8.4 ± 2.2	7.9 ± 1.8	9.0 ± 3.1
Resorption	0.3 ± 0.6	0.3 ± 0.7	0.2 ± 0.4	0.2 ± 0.7
Does with any resorptions	7 (30.4%)	5 (21.7%)	3 (20%)	1 (12.5%)
Live male foetuses	104	84	59	30
Live foetal body weight (g)	42.31 ± 5.33	42.19 ± 5.80	43.90 ± 5.50	38.15 ± 7.22
Body weight: live male foetuses/litter	43.20 ± 5.56	43.21 ± 6.07	44.98 ± 5.74	**36.60 ± 6.80***
Body weight: live female foetuses/litter	41.16	41.60	43.05	38.25

Statistically significant: * = $p < 0.05$; ** = $p < 0.01$;

Table 2.6.6.2.1-10: Foetal soft tissue and skeletal alterations

Dose / Findings	0 (mg/kg bw/day)	250 (mg/kg bw/day)	500 (mg/kg bw/day)	1000 (mg/kg bw/day)
Litter evaluated	23	23	15	8
Foetuses evaluated	195	194	119	72
Foetuses with any alteration observed	13 (6.7 %)	17 (8.8 %)	12 (10.1%)	**14 (19.4%)****
Foetuses with any alteration/litter (%)	7.3 ± 9.7	8.7 ± 11.4	10.8 ± 12.1	20.0 ± 17.0
Soft tissue-litter incidence				
Heart: Interventricular septal defect	0 (0%)	1 (4.3%)	0 (0%)	0 (0%)
Vessels: Aorta distended	0 (0%)	1 (4.3%)	0 (0%)	0 (0%)
Vessels: Pulmonary artery constricted	0 (0%)	1 (4.3%)	0 (0%)	0 (0%)
Skeletal alterations-litter incidence				
Scull: Irregular ossification	5 (21.7%)	6 (26.1%)	6 (40.0%)	5 (62.5%)
Cervical vertebrae: Cervical rib present at 7th cervical vertebra	0 (0%)	1 (4.3%)	0 (0%)	0 (0%)
Thoracic vertebrae: Hemivertebra	0 (0%)	1 (4.3%)	1 (6.7%)	0 (0%)
Thoracic vertebrae: Centrum, bifid	0 (0%)	1 (4.3%)	0 (0%)	0 (0%)
Sacral vertebrae: Fused	0 (0%)	0 (0%)	0 (0%)	1 (12.5%)
Caudal vertebrae: Fused	0 (0%)	1 (4.3%)	0 (0%)	1 (12.5%)
Caudal vertebrae: 13 present	0 (0%)	0 (0%)	0 (0%)	1 (12.5%)
Caudal vertebrae: Misaligned	2 (8.7%)	1 (4.3%)	1 (6.7%)	2 (25.0%)
Ribs: Thickened	2 (8.7%)	1 (4.3%)	0 (0%)	1 (12.5%)

Ribs: Thickened-Foetal incidence	2 (1.0%)	1 (0.5%)	0 (0%)	**5 (6.9%)****
Manubrium: irregularly shaped	0 (0%)	0 (0%)	1 (6.7%)	0 (0%)
Sternal centra: Fused	1 (4.3%)	0 (0%)	2 (13.3%)	0 (0%)
Sternal centra: Incompletely ossified	0 (0%)	1 (4.3%)	1 (6.7%)	0 (0%)
Sternal centra: Asymmetric	0 (0%)	0 (0%)	1 (6.7%)	0 (0%)
Xipnoid: Irregularly shaped	0 (0%)	0 (0%)	1 (6.7%)	0 (0%)
Scapulae: Ala, angulated	0 (0%)	0 (0%)	0 (0%)	1 (12.5%)

Statistically significant: * = $p < 0.05$; ** = $p < 0.01$;

Table 2.6.6.2.1-11: Foetal ossification sites

Dose / **Findings**	**0 (mg/kg bw/day)**	**250 (mg/kg bw/day)**	**500 (mg/kg bw/day)**	**1000 (mg/kg bw/day)**
Forelimb-phalanges	13.99 ± 0.04	13.82 ± 0.27	13.85 ± 0.37	13.65 ± 0.27**

Statistically significant: ** = $p < 0.01$;

In the rabbit study *(Anonymous, 1985)*, treatment-induced differences in maternal appearance and behaviour included the death of a 300 mg/kg bw/day doe on gestation day 12 and an increased incidence of diarrhoea, soft, small amount, or absent stool across the treated groups. Since the observed stool changes were not found to be of toxicological relevance, the NOAEL for maternal toxicity was established at ≥ 300 mg/kg bw/day. Caesarean section and foetal morphological observations were not affected at any tested dose level. Therefore, the NOAEL for developmental toxicity was established at ≥ 300 mg/kg bw/day. The test material did not induce a teratogenic effect in Dutch Belted rabbits up to the dose level of 300 mg/kg bw/day.

Table 2.6.6.2.1-10: Caesarean-sectioning and litter observations; *(Anonymous, 1985)*

Dose / **Findings**	**0 (mg/kg bw/day)**	**50 (mg/kg bw/day)**	**150 (mg/kg bw/day)**	**300 (mg/kg bw/day)**
Pregnant	13 (81%)	14 (88%)	16 (100%)	10 (63%)
Found dead	0	0	0	1
Moribund sacrificed	1 (4%)	1 (4%)	2 (8%)	6 (25%)
Aborted and sacrificed	1	0	1	1
No. of caesarean-sectioned animal	15	16	15	14
Corpora lutea	12.6 ± 3.15	10.6 ± 3.37	11.1 ± 2.30	10.6 ± 3.25
Does with viable foetuses	12	13	14	8
Viable foetuses/doe	7.1 ± 3.37	6.9 ± 3.02	6.8 ± 3.03	6.9 ± 3.40
Implantation/doe	8.1 ± 3.26	8.3 ± 2.67	8.3 ± 2.55	8.5 ± 4.11
Preimplantation loss (%)	35.8	21.6	20.6	20.0
Post-implantation loss (%)	12.4	16.4	18.4	19.1
Does with resorptions only	0	1	1	0
Live foetal body weight (g)	32.3 ± 5.27	32.9 ± 4.27	32.3 ± 4.86	31.5 ± 10.68
Sex ratio (males/females, %)	54.1/45.9	54.6/45.4	53.9/46.1	42.9/57.1

Table 2.6.6.2.1-11: Foetal alterations

Dose / Findings	0 (mg/kg bw/day)	50 (mg/kg bw/day)	150 (mg/kg bw/day)	300 (mg/kg bw/day)
Litter evaluated	12	13	14	8
Foetuses evaluated	85	97	102	56
Foetuses with any alteration observed	5 (5.9%)	5 (5.2 %)	3 (2.9%)	3 (5.4%)
Ethmocephaly	1 (1.2%)	0 (0%)	0 (0%)	0 (0%)
Exencephaly	0 (0%)	1 (1.0%)	0 (0%)	0 (0%)
Ablepharia	0 (0%)	1 (1.0%)	0 (0%)	0 (0%)
Cleft palate	0 (0%)	1 (1.0%)	0 (0%)	0 (0%)
Hydrocephaly	1 (1.2%)	0 (0%)	0 (0%)	1 (1.8%)
Omphalocele	0 (0%)	1 (1.0%)	0 (0%)	1 (1.8%)
Spleen absent	0 (0%)	0 (0%)	0 (0%)	1 (1.8%)
Fused skull bones	1 (1.2%)	2 (2.1%)	2 (2.0%)	0 (0%)
Forked scapula	2 (2.4%)	2 (2.1%)	0 (0%)	1 (1.8%)
Vertebral anomaly	0 (0%)	0 (0%)	1 (1.0%)	0 (0%)

2.6.6.2.2 Comparison with the CLP criteria regarding adverse effects on development

According to CLP criteria (Regulation (EC) No. 1272/2008), the major manifestations of developmental toxicity include (1) death of the developing organism, (2) structural abnormality, (3) altered growth, and (4) functional deficiency. In the studies with daminozide, the increased incidence of abortions, decreased foetal weight, and reduced ossification occurred in rabbits in the presence of maternal toxicity (excessive mortality, hyperactivity, hyperpnoea, convulsions, decreased food consumption) at the top dose of 1000 mg/kg bw/day, which is, however, according to OECD TG 414 recommended as the limit dose. According to CLP regulation adverse effects on reproduction seen only at very high dose levels in animal studies (for example doses that induce prostration, severe inappetence, excessive mortality) would not normally lead to classification. Moreover, these observation were not proved in the rat studies (with the exception of non-significant decrease in foetal body weight by 3% in the presence of maternal toxicity: reduced bw gain by 35.5 and 11.9% during weeks 6 – 9 and 6 – 15, respectively). According to CLP regulation the reduction in foetal/pup body weight or retardation of ossification associated with maternal toxicity is not the reasonfor classification. No indications of any teratogenic potential of daminozide were observed.

2.6.6.3 Adverse effects on or via lactation [equivalent to section 10.10.7 of the CLH report template]

Table 51: Summary table of animal studies on effects on or via lactation

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference	Type of data/report
No data available					

Table 52: Summary table of human data on effects on or via lactation

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference	Type of data/report
No data available					

Table 53: Summary table of other studies relevant for effects on or via lactation¨

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference	Type of data/report
No data available					

2.6.6.3.1 Short summary and overall relevance of the provided information on effects on or via lactation

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference	Type of data/report
No data available					

2.6.6.3.2 Comparison with the CLP criteria regarding effects on or via lactation

No data available.

2.6.6.4 Conclusion on classification and labelling for reproductive toxicity

Based on the results of generation and teratogenicity studies, daminozide does not meet CLP criteria (Regulation (EC) No. 1272/2008) for classification.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

The DS proposed no classification for effects on sexual function and fertility based on information from two two-generation studies in rats performed according to OECD TG 416. Both studies reported no adverse effects on the reproductive function (mating, fertility, and length of gestation, litter size, sex ratio and pup's survival) up to dose levels of 1200 mg/kg bw/day. No treatment-related clinical observations or mortality were noted in parental animals or pups during the studies. Teratogenicity studies with daminozide are available in rabbits (two studies) and rats (two studies). An increased incidence of abortions, decreased foetal weight, and reduced ossification were reported in rabbits, however in the presence of excessive maternal toxicity (mortality, convulsions) at a limit dose of 1000 mg/kg bw/day. No classification for developmental toxicity was proposed.

Comments received during public consultation

One MSCA and one manufacturer supported no classification for reproductive toxicity.

Assessment and comparison with the classification criteria

Sexual function and fertility

Two reproduction toxicity studies according to OECD TG 416 are available for assessment of this endpoint.

In a dietary two-generation reproduction study (Anonymous, 1987), Crl:CD (SD)BR rats (25/dose/sex) were treated with Alar® (purity 99%) at dose levels of 0, 100, 1000 and 10000 ppm (corresponding to 0, 5, 50 and 500 mg/kg bw/day, respectively) for 10 weeks prior mating, and for females during mating, pregnancy and lactation. F1 animals were fed on the same diet for 10 weeks post-weaning, and mated to produce F2 generation. In deviation from the current guideline, sperm parameters, age of puberty attainment, and organ weights at the time of termination were not evaluated.

No treatment-related mortalities or adverse clinical observations were noted in P0 and F1 animals. A statistically significant ($p \leq 0.5$) decrease of body weight was observed only in F1 males of the top dose group (-8.3%). Fertility, mating, days required to mate and length of gestation were not affected in any of the P0 and F1 groups. No differences were noted for the number of pups born alive, litter size, pup survival, pup weights, pup weight gains or sex ratio in pups from both P0 and F1 dams. Significantly lower body weights on day 7 and weight gains on days 4 through 7 were reported for pups from F1 dams treated with 100 ppm. However, these differences were not consistent and without a dose-response.

In a second two-generation reproduction study (Anonymous, 1994), OFA(SD)IOPS-Caw rats (a Sprague Dawley derived strain, 30/dose/sex) were given daminozide (purity >99%) by gavage at dose levels of 0, 60, 360 or 1200 mg/kg bw/day, beginning 10 weeks before mating and throughout the study. Premature deaths of two control females and one low dose male from the P0 generation as well as one male and one female from the low dose group of F1

generation were not considered treatment related. Clinical signs such as loose and/or odorous faeces, perianal fur staining and post-dose salivation in parental animals were noted in both generations at 1200 mg/kg bw/day. No treatment-related effects were reported in the parental animals of the low and mid dose groups. Mating, fertility, litter size and pup's survival, weight and clinical condition were not affected at any dose level. RAC concludes that both two-generation studies do not provide any evidence for adverse effects and agrees with DS assessment that **no classification for sexual function and fertility is justified.**

Adverse effects on development

Three guideline prenatal developmental toxicity studies (one in rats and two in rabbits) are available for assessment of this endpoint. In addition, information from a preliminary dose range finding study in rabbits and a non-guideline teratogenicity study in rats from the open literature is considered.

In a prenatal developmental toxicity study according to OECD TG 414 (Anonymous, 1993), 25 pregnant Sprague Dawley rats were exposed once daily by gavage to daminozide (purity >99%) at dose levels of 0, 150, 750, and 1500 mg/kg bw/day during days 6-15 of gestation (GD). No treatment-related mortalities, adverse changes in clinical condition, or abnormalities in pathology were reported at necropsy. Body weight gain during GD 6-9 was significantly reduced at 750 and 1500 mg/kg bw/day by 31% and 35%, respectively. Statistically significantly lower bodyweight gain (12%) was also reported for the entire treatment period at the top dose. The corrected maternal body weights were not affected.

There were no effects on numbers of implantations, live foetuses or sex ratio at any dose level. Compared to controls, mean foetal weights were slightly lower (3%, not statistically significant) at 750 and 1500 mg/kg bw/day, and slightly higher than control in the low dose group. The effect at mid and high dose was attributed to the larger litter size and reduced maternal body weight gain in these groups. Major abnormalities were observed only in two foetuses at the lowest dose (one with cleft palate and one with umbilical hernia). Since no further major abnormalities were observed at the higher dose levels, these effects were considered to be spontaneous. No adverse effects on development such as external, visceral or skeletal abnormalities or incidences of foetuses with variants were noted. The report concluded that there were no signs of embryotoxicity or teratogenicity. As clarified by DS, the term major abnormalities used in the original report corresponds to malformations, whereas minor abnormalities to the "grey zone" (i.e. no consensus whether it should be regarded as a variation or a malformation) or variations.

In a teratogenicity study by Khera (1979), groups of 20 mated female rats were given daminozide (purity >99%) by oral gavage at doses of 0, 300, 600, and 1000 mg/kg bw/day on GD 6-15. No signs of toxicity were reported, and pregnancy rates, numbers of corpora lutea, implantation and resorption rates, foetal deaths, sex ratio, foetal weights, number of live foetuses, as well as the incidence of foetal anomalies and skeletal malformations were not significantly different from control values. This study is considered supplementary since it is not GLP or guideline compliant, and no individual data were presented.

In a prenatal developmental toxicity study according to OECD TG 414 (Anonymous, 2006b), time-mated female Hra:(NZW)SPF rabbits (25/group) were administered daminozide via oral gavage on days 6 through 28 of gestation at doses of 0, 250, 500 and 1000 mg/kg bw/day. 7 and 8 animals were found death and 2 and 6 animals were early sacrificed at dose levels of 500 and 1000 mg/kg bw/day, respectively (Table below). In addition, two animals at the top

dose aborted and were sacrificed. The mortality (except for one animal of each group) and the abortions were considered treatment-related. Clinical signs including soft or liquid faeces, ungroomed coat, hyperactivity, perinasal or perioral substance, hyperpnoea, convulsions, tremors, impaired righting reflex, and gasping were reported in both the mid and top dose groups. In the 1000 mg/kg bw/day group, the number of animals with scant faeces, mucoid faeces, decreased motor activity, dehydration, dyspnoea, ptosis, blue or light blue colouring around the mouth and cold to touch was significantly increased. Compared to controls, body weight gain during the entire exposure period was reduced by ca. 21% at 1000 mg/kg bw/day, however corrected body weights did not differ significantly among the groups.

Foetal weights were reduced by 10% in the 1000 mg/kg bw/day group (in male foetuses by 15.3%) when compared to controls. The number of foetuses with alterations was significantly increased in the 1000 mg/kg bw/day group. This included a significant increase in the number of foetuses with thickened ribs and a decrease in the average number of ossified forelimb phalanges. No other gross external, soft tissue or skeletal malformations/variations caused by the test substance were reported. It is noted that the decreased foetal weight as well as reduced ossification were observed at a dose of excessive maternal toxicity (mortality of >50% and clinical observations). According to the guideline, groups with mortality rate in dams >10%, dose are not normally considered for further evaluation.

***Table**: Clinical observation and selected foetal examination parameters (Anonymous, 2006b)*

Dose (mg/kg bw/day)	**0**	**250**	**500**	**1000**
Animals per group	24	24	24	24
Overall mortality	1	1	9*	14**
Found dead	0	0	7 (29%)**	8 (33%)**
Moribund sacrificed	1 (4%)	1 (4%)	2 (8%)	6 (25%)
Aborted and sacrificed	0	0	0	2 (8%)
Clinical observations				
Hyperactivity	0	0	3*	5**
Convulsion	0	0	3	3
Tremors	0	0	1	3
Ptosis	0	0	0	4**
Ungroomed coat	4	5	14	24**
Scant faeces	3	5	5	16**
Mucoid faeces	0	1	2	12**
Foetal examination				
Litters (foetuses) evaluated	23 (195)	23 (194)	15 (119)	8 (72)
Foetuses with any alteration observed	13 (6.7 %)	17 (8.8 %)	12 (10.1%)	14 (19.4%)**
Scull: Irregular ossification	5 (21.7%)	6 (26.1%)	6 (40.0%)	5 (62.5%)
Ribs: Thickened (litter incidence)	2 (8.7%)	1 (4.3%)	0 (0%)	1 (12.5%)
Ribs: Thickened (foetal incidence)	2 (1.0%)	1 (0.5%)	0 (0%)	5 (6.9%)**
Ossified forelimb phalanges	13.99±0.04	13.82±0.27	13.85±0.37	13.6±0.27**

*p<0.05, **p<0.01;

In a second prenatal developmental study according to OECD TG 414, inseminated Dutch Belted rabbits (16/dose) received Alar® (purity >99%) by oral gavage at doses of 0, 50, 150, and 300 mg/kg bw/day, on days 7 through 19 of gestation (Anonymous, 1985). One dam of the top dose group died on gestation day 12, and the cause of death was undetermined. Ante-mortem and necropsy observations included ocular and nasal discharge, wet/matted hair coat (eyes, nose, mouth, ventral neck, forelimbs), small amount of stool, a thick white substance in the pan beneath the cage, and congested lungs. Three females aborted (one each in the control, mid and top dose group) and were subsequently sacrificed. Clinical observations included increased incidence of diarrhoea, soft, small amount, or absent stool across all treated groups. Caesarean section parameters and foetal morphological observations were not affected at any tested dose level. The study concluded that the test substance did not induce a teratogenic effect in Dutch Belted rabbits up to the dose level of 300 mg/kg bw/day.

***Table**: Clinical observation and selected foetal examination parameters (Anonymous, 1985)*

Dose (mg/kg bw/day)	**0**	**50**	**150**	**300**
Animals per group	16	16	16	16
Pregnant	13 (81%)	14 (88%)	16 (100%)	10 (63%)
Found dead	0	0	0	1 (6.3%)
Moribund sacrificed	1 (4%)	1 (4%)	2 (8%)	6 (25%)
Aborted and sacrificed	1 (6.3%)	0	1 (6.3%)	1 (6.3%)
Foetal examination				
Litter (foetus) evaluated	12 (85)	13 (97)	14 (102)	8 (56)
Foetuses evaluated	85	97	102	56
Foetuses with any alteration observed	5 (5.9%)	5 (5.2 %)	3 (2.9%)	3 (5.4%)
Ethmocephaly	1 (1.2%)	0 (0%)	0 (0%)	0 (0%)
Exencephaly	0 (0%)	1 (1.0%)	0 (0%)	0 (0%)
Ablepharia	0 (0%)	1 (1.0%)	0 (0%)	0 (0%)
Cleft palate	0 (0%)	1 (1.0%)	0 (0%)	0 (0%)
Hydrocephaly	1 (1.2%)	0 (0%)	0 (0%)	1 (1.8%)
Omphalocele	0 (0%)	1 (1.0%)	0 (0%)	1 (1.8%)
Spleen absent	0 (0%)	0 (0%)	0 (0%)	1 (1.8%)
Fused skull bones	1 (1.2%)	2 (2.1%)	2 (2.0%)	0 (0%)
Forked scapula	2 (2.4%)	2 (2.1%)	0 (0%)	1 (1.8%)

Overall, the study provides little support for classification purpose because the top dose tested appears too low as indicated by very mild maternal toxicity.

As a summary on developmental toxicity, in the studies with daminozide, increased incidence of abortions, decreased foetal weight, and reduced ossification were observed in rabbits at the top dose of 1000 mg/kg bw/day (limit dose), in the presence of severe maternal toxicity (excessive mortality of >50%, hyperactivity, hyperpnoea, convulsions, decreased food consumption). According to the CLP Regulation, dose groups with mortality rate in dams >10% should not normally be considered for further evaluation. In addition, no similar findings were observed in the rat studies with dose levels of up to 1500 mg/kg bw/day. RAC agrees with the conclusion of DS that no indications of teratogenic potential of daminozide were observed in the available studies and therefore **no classification for developmental toxicity is**

warranted.

Adverse effects on or via lactation

The DS proposal for no classification due to lack of data was agreed by RAC.

2.6.7 Summary of neurotoxicity

Table 54: Summary table of animal studies on neurotoxicity

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results:** **- NOAEL/LOAEL** **- target tissue/organ** **-critical effect at LOAEL**	**Reference**
Acute neurotoxicity study OECD 424 Rats (Crl:CD(SD)) Females, Males 10 animals/group **Acceptable study**	daminozide Purity: 100% Oral: by gavage; a single dose Dose levels: 0, 500, 1000, 2000 mg/kg bw/day Vehicle: 0.5% carboxymethylcellulose Observation period: 14 days	NOAEL: 1000 mg/kg bw/day LOAEL: 2000 mg/kg bw/day Critical effect: decreased locomotor activity (total distance, basic and fine movement; *see Table 2.6.7-1)*	*Anonymous (2012a*)
90-day oral neurotoxicity study OECD 424 Rats (Crl:CD(SD)) Females, Males 10 animals/group **Acceptable study**	daminozide Oral: by gavage for 90 days Dose levels: 0, 100, 300, 1000 mg/kg bw/day Vehicle: 0.5% carboxymethylcellulose Purity: 100%	NOAEL: 1000 mg/kg bw/day (top dose) No signs of systemic toxicity and neurotoxicity were observed	*Anonymous (2012b)*

Two neurotoxicity studies in rats are available. The NOAEL for neurotoxicity derived from the acute neurotoxicity study *(Anonymous, 2012a)* was set at 1000 mg/kg bw/day based on the decreased locomotor activity (total distance, basic and fine movement on Day 1 and 14 in males; on Day 14 in females) over the entire 0 – 60 minute collection period in rats of the top dose group (2000 mg/kg bw/day) when compared to the control *(see Table 2.6.7-1)*. The NOAEL for neurotoxicity derived from subchronic neurotoxicity study *(Anonymous, 2012b)* was established at 1000 mg/kg bw/day (top dose). No treatment-related neuropathological lesions were observed.

Table 2.6.7-1: Summary of locomotor activity (0-60 minute study interval); *(Anonymous, 2012a)*

Dose [mg/kg bw/day]	**0**		**500**		**1000**		**2000**	
Parameter	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
Basic movement (count)								
Pre-test	4207.9	3629.0	3941.0	3648.6	4181.5	3918.0	3864.8	3646.9
Day1	2316.2	2984.8	2269.7	2817.9	2486.2	2645.4	1683.2	2790.2
Day7	3373.6	4065.4	3447.3	3902.7	3635.3	4034.2	3168.6	4136.9
Day14	4757.4	5286.0	4730.8	4358.6	4151.3	4474.2	3691.2	3250.9
Fine movement (count)								
Pre-test	3282.4	2698.7	3094.5	2651.7	3257.2	2878.0	2975.1	2697.7
Day1	1873.0	2135.3	1872.8	2067.8	1987.2	1997.9	1322.3	2099.2
Day7	2758.9	2992.1	2832.1	2999.5	2942.4	3014.1	2608.8	3115.2
Day14	3798.3	3864.4	3753.8	3321.4	3302.4	3275.1	2923.2	2476.8
Rearing (count)								
Pre-test	184.8	115.3	175.1	115.4	188.0	129.8	148.4	117.1
Day1	98.6	93.9	112.6	96.9	107.0	90.9	63.5	84.0
Day7	194.8	138.6	218.9	145.7	208.3	135.2	175.2	139.6
Day14	286.9	217.6	273.7	179.2	228.3	176.4	217.7	126.2*
Total distance (cm)								
Pre-test	7488.8	6362.5	6901.0	6522.6	7391.8	6862.9	6893.7	6359.4
Day1	4024.4	5339.1	3920.1	4919.9	4369.1	4635.9	3029.0	4892.6
Day7	5898.3	7152.1	6058.2	6827.7	6359.3	7014.0	5575.5	7297.7
Day14	8304.9	9319.8	8195.2	7623.5	7240.3	7785.4	6533.5	5685.7*

2.6.8 Summary of other toxicological studies

2.6.8.1 Toxicity studies of metabolites and impurities

Daminozide is converted by hydrolysis to 1, 1-dimethylhydrazine (UDMH), which is subsequently oxidized to N-nitrosodimethylamine (NDMA); (see Figure 2.6.1.1-1). Subchronic, genotoxicity and carcinogenicity studies on UDMH were provided.

Genotoxicity studies:

Bacterial reverse mutation assay (Ames test): UDMH did not induce mutations in *Salmonella typhimurium* strains either in the presence or absence of the metabolic activation *(Stankowski, 1986)*. However, the strain for detection of oxidizing and cross-linking agents was not used and the purity of the test substance was not stated. Ames test with *Escherichia coli* was not performed.

In vitro mammalian cell gene mutation assay (HPRT test): The results of HPRT test with CHO cells were equivocal in the first study *(Stankowski 1987)*, whereas negative in the second one *(Stankowski 1988)*. However, each of these studies serves only as a supplementary material because the purity of UDMH was not stated.

I*n vitro* chromosome aberration assay: This test (*San Sebastian, 1986*) was performed with several deviations from OECD TG 473, e.g. the long-term treatment was not performed, the purity of the test substance was not stated, therefore is regarded as a supplementary material. Nevertheless, under conditions of the study showed a negative result.

DNA damage and repair, unscheduled DNA synthesis in mammalian cells *in vitro*: UDMH was found negative in an *in vitro* UDS assay with rat hepatocytes *(Barfknecht, 1986)*. OECD TG for this test was deleted in 2014.

In vivo genotoxicity studies: not provided

Literature data: UDMH was found positive in an *in vivo* mouse-liver micronucleus test *(Cliet, 1989)*. In a covalent binding study, UDMH and NDMA were found to bind to DNA of the liver *(Sagelsdorff, 1988)*.

The extent of DNA damage expressed as the covalent binding index (CBI; µmol chemical bound per mol nucleotide/mmol chemical applied per kg body weight), values of 0.55, 26 and 2700 were counted for daminozide, UDMH and NDMA, respectively. Compounds with CBI: (i) > 1000 are regarded as potent carcinogens; (ii) of the order of 100 as moderately strong carcinogens; (iii) < 10 weakly genotoxic carcinogens; If the CBI < 1, it is unlikely that the substance will induce tumours via DNA binding (*Sagelsdorff, 1988*; *see 2.6.4.1*). Therefore, based on the data of this study, the genotoxic potential of UDMH cannot be excluded without any doubt, whereas NDMA can be regarded as a potent carcinogen.

90-day oral toxicity studies: 90-day toxicity study in rats as well as mice is available. However, the both studies represent only supplementary material because the purity of UDMH was not stated. In rats *(Anonymous, 1987a)*, no treatment-related toxic effects were observed. The NOAEL was set at the highest dose tested (125 ppm equal to 8.98 mg/kg bw/day).

In mice *(Anonymous, 1987b)*, liver hypertrophy, karyomegaly, and accentuation of lobulation occurred in all treated male groups (already at the lowest dose of 10 ppm equal to 2 mg/kg bw/day; *see Table 2.6.8.1-1*), therefore NOAEL could not be derived from this study. Moreover, alveolar/bronchial adenomas were observed at the two highest doses of 100 (in females) and 250 ppm (in males); *see Table 2.6.8.1-2*. This finding was considered to be treatment-related based on: (i) the results of 2-year oncogenicity studies in mice (*Anonymous, 1989b; Anonymous, 1990; see the summary bellow*), clearly demonstrating the carcinogenic potential of UDMH; (ii) the fact that the occurrence of

alveolar/bronchiolar adenomas during the subchronic studies is rather rare. Thus, results of this study indicate that UDMH decreases the latency period of lung tumours.

Table 2.6.8.1-1: Macroscopic and microscopic findings in the liver *(Anonymous, 1987b)*

Dose[ppm]	Males (10 animals)					Females (10 animals)				
	0	**10**	**25**	**100**	**250**	**0**	**10**	**25**	**100**	**250**
Accentuation of liver lobulation										
Mild	**0**	**4**	**4**	**2**	**2**	**0**	**0**	**1**	**5**	**2**
Moderate	**0**	**0**	**5**	**8**	**8**	**0**	**0**	**0**	**0**	**1**
Brown pigment										
Trace	**0**	**0**	**4**	**3**	**1**	**0**	**0**	**0**	**6**	**8**
Mild	**0**	**0**	**2**	**6**	**5**	**0**	**0**	**0**	**1**	**1**
Moderate	**0**	**0**	**0**	**1**	**4**	**0**	**0**	**0**	**0**	**0**
Karyomegaly/Hyperthrophy										
Trace	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Mild	**0**	**2**	**1**	**4**	**7**	**0**	**1**	**0**	**0**	**1**
Moderate	**0**	**0**	**0**	**1**	**2**	**0**	**0**	**0**	**0**	**0**

Table 2.6.8.1-2: Incidence of alveolar/bronchiolar adenomas *(Anonymous, 1987b)*

Dose[ppm]	Males (10 animals)					Females (10 animals)				
	0	**10**	**25**	**100**	**250**	**0**	**10**	**25**	**100**	**250**
Alveolar/bronchiolar adenomas	**0**	**0**	**0**	**0**	**2**	**0**	**0**	**0**	**1**	**0**

Carcinogenicity studies: 2-year chronic carcinogenicity studies with UDMH were conducted in rats and mice (high and low dose level; purity of the test substance not stated). In rats (*Anonymous, 1989a),* the increased incidence of hepatocellular neoplasms was observed in females at all dose levels (0.1 – 8 mg/kg bw/day); *see Table 2.6.8.1-3*.. Therefore, only provisional NOAEL at 0.1mg/kg bw/day was established. In all dose groups, hepatocellular neoplasms were associated with chronic inflammation of the liver.

Table 2.6.8.1-3: Tumour incidence (overall rate); (*Anonymous, 1989a);* *p≤0.05, ** p≤0.01, ***p≤0.001 (Fisher exact test)

Dose[ppm]	Males				Females			
	0	**1**	**50**	**100**	**0**	**1**	**50**	**100**
LIVER								
Hepatocellular adenoma	2/70 (2.9%)	0/70 (0%)	1/70 (1.4%)	2/70 (2.9%)	0/70 (0%)	1/70 (1.4%)	2/70 (2.9%)	1/70 (1.4%)

Hepatocellular carcinoma	1/70 (1.4%)	0/70 (0%)	0/70 (0%)	1/70 (1.4%)	0/70 (0%)	0/70 (0%)	3/70 (4.3%)	4/70 (5.7%)
Hepatocellular adenoma/carcinoma	3/70 (4.3%)	0/70 (0%)	1/70 (1.4%)	3/70 (4.3%)	0/70 (0%)	1/70 (1.4%)	5/70* (7.1%)	5/70* (7.1%)
ADRENAL								
Pheochromocytoma (benign)	3/70 (4.3%)	5/70 (7.1%)	1/70 (1.4%)	6/70 (8.6%)	-	-	-	-
HAEMOLYMPHORETICULAR SYSTEM								
Mononuclear cell leukemia	33/70 (47.1%)	21/70* (30.0%)	21/70* (30.0%)	16/70** (22.9%)	21/70 (30.0%)	18/70 (25.7%)	8/70** (11.4%)	10/70* (14.3%)
MAMMARY REGION								
Fibroadenoma	-	-	-	-	6/70 (8.6%)	5/70 (7.1%)	5/70 (7.1%)	3/70 (4.3%)
PITUITARY								
Pituitary adenoma	16/70 (22.9%)	15/70 (21.4%)	18/70 (25.7%)	13/70 (18.6%)	17/69 (24.6%)	23/69 (33.3%)	19/70 (27.1%)	32/70** (45.7%)
SKIN								
Fibroma	3/70 (4.3%)	2/70 (2.9%)	3/70 (4.3%)	2/70 (2.9%)	-	-	-	-
TESTIS								
Interstitial cell tumour (benign)	47/70 (67.1%)	42/70 (60.0%)	50/70 (71.4%)	45/70 (64.3%)	-	-	-	-
THYROID								
Parafollicular cell adenoma	6/70 (8.6%)	6/70 (8.6%)	3/70 (4.3%)	6/70 (8.6%)	2/70 (2.9%)	4/70 (5.7%)	3/70 (4.3%)	4/70 (5.7%)
UTERUS								
Polyp	-	-	-	-	9/70 (12.9%)	9/70 (12.9%)	4/70 (5.7%)	10/70 (14.3%)

In the mouse study (*Anonymous, 1989b)*, conducted with lower levels (ranging from 0.2 – 2.7 mg/kg bw/day), the incidence of alveolar/bronchiolar adenomas and carcinomas was significantly increased at the top dose females *(see Table 2.6.8.1-4)*. As for non-neoplastic lesions, the incidence of brown pigment in the liver was increased in the treated mice from the dose of 5 ppm (equal to 1.41 mg/kg bw/day). Special stains were not performed to determine the type of the pigment. The mixture seemed to consist predominantly of lipofuscin, which is associated with aging in many organs. A part of the pigmentation seemed to be formed by bile pigment, which is indicative of hepatotoxicity.

Table 2.6.8.1-4: Tumour incidence (overall rate); (*Anonymous, 1989b*); *p≤0.05, ** p≤0.01, ***p≤0.001 (Fisher exact test)

Dose[ppm]	Males				Females			
	0	**1**	**5**	**10**	**0**	**1**	**5**	**20**
LIVER								
Hepatocellular adenoma	12/90 13.3%	5/90 5.6%	7/90 7.8%	13/90 14.4%	4/90 4.4%	1/90 1.1%	0/90 0%	4/90 4.4%
Hepatocellular carcinoma	5/90 5.6%	2/90 2.2%	5/90 5.6%	8/90 8.9%	1/90 1.1%	0/90 0%	0/90 0%	1/90 1.1%
Hepatocellular adenoma/carcinoma	17/90 18.9%	7/90* 7.8%	12/90 13.3%	21/90 23.3%	5/90 5.6%	1/90 1.1%	0/90* 0%	5/90 5.6%
Haemangiosarcoma	0/90 0%	1/90 1.1%	0/90 0%	2/90 2.2%	3/90 3.3%	2/90 2.2%	1/90 1.1%	5/90 5.6%
LUNG								
Alveolar/bronchiolar adenoma	16/90 17.8%	14/90 15.6%	19/90 21.1%	16/90 17.8%	9/90 10.0%	13/89 14.6%	16/90 17.8%	24/90** 26.7%
Alveolar/bronchiolar carcinoma	4/90 4.4%	4/90 4.4%	7/90 7.8%	4/90 4.4%	1/90 1.1%	1/89 1.1%	1/90 1.1%	7/90* 7.8%
Alveolar/bronchiolar adenoma/carcinoma	20/90 22.2%	18/90 20.0%	26/90 28.9%	20/90 22.2%	10/90 11.1%	14/89 15.7%	17/90 18.9%	31/90*** 34.4%
HAEMOLYMPHORETICULAR SYSTEM								
Malignant lymphoma (lymphocytic)	1/90 1.1%	0/90 0%	0/90 0%	2/90 2.2%	3/90 3.3%	1/90 1.1%	5/90 5.6%	2/90 2.2%
Histiocytic sarcoma	0/90 0%	0/90 0%	1/90 1.1%	0/90 0%	2/90 2.2%	3/90 3.3%	1/90 1.1%	2/90 2.2%
UTERUS								
Polyp	-	-	-	-	3/90 3.3%	2/71 2.8%	0/76 0%	5/90 5.6%
Haemangioma	-	-	-	-	1/90 1.1%	4/71 5.6%	0/76 0%	2/90 2.2%

In the second mouse study (*Anonymous, 1990)* conducted with higher dose levels (40 and 80 ppm equal to 7.3 and 21.8 mg/kg bw/day, respectively), the significant increase in the incidence of neoplastic lesions in the liver

(haemangiomas/haemagiosarcomas) and lung (alveolar/bronchiolar adenomas/carcinomas) was observed at all male and female treated groups *(see Table 2.6.8.1-5).* Other treatment-related effects included: decreased animal survival *(see Table 2.6.8.1-8)* and hepatotoxicity (accentuated liver lobulation, liver cell hyperthrophy and necrosis, presence of chronic inflammation and brown pigment, elevated levels of alanine aminotransferase and sorbitol dehydrogenase; *see Table 2.6.8.1-6 and 2.6.8.1-7).* However, the excessive mortality in this study indicates that the dosing was probably set over the maximum tolerated dose (MTD).

Table 2.6.8.1-5: Tumour incidence (overall rate); (*Anonymous, 1990*); *p≤0.05, ** p≤0.01, ***p≤0.001 (Fisher exact test)

Dose[ppm]	**Males**			**Females**		
	0	**40**	**80**	**0**	**40**	**80**
KIDNEY						
Adenoma (cortical)	0/90 (0%)	0/90 (0%)	3/90 (3.3%)	1/90 (1.1%)	0/90 (0%)	0/90 (0%)
LIVER						
Hepatocellular adenoma	7/90 (7.8%)	8/90 (8.9%)	10/90 (11.1%)	2/89 (2.2%)	6/90 (6.7%)	1/90 (1.1%)
Hepatocellular carcinoma	3/90 (3.3%)	* 11/90 (12.2%)	0/90 (0%)	0/90 (0%)	0/90 (0%)	0/90 (0%)
Hepatocellular adenoma/carcinoma	10/90 (11.1%)	19/90 (21.1%)	10/90 (11.1%)	2/89 (2.2%)	6/90 (6.7%)	1/90 (1.1%)
Haemangioma	0/90 (0%)	2/90 (2.2%)	2/90 (2.2%)	1/89 (1.1%)	2/90 (2.2%)	2/90 (2.2%)
Haemangiosarcoma	4/90 (4.4%)	*** 29/90 (32.2%)	*** 39/90 (43.3%)	1/89 (1.1%)	** 10/90 (11.1%)	*** 38/90 (42.2%)
Liver haemangioma/ haemangiosarcoma	4/90 (4.4%)	*** 31/90 (34.4%)	*** 41/90 (45.6%)	2/89 (2.2%)	** 12/90 (13.3%)	*** 40/90 (44.4%)
LUNG						
Alveolar/bronchiolar adenoma	22/90 (24.4%)	* 35/90 (38.9%)	** 38/90 (42.2%)	17/89 (19.1%)	* 31/90 (34.4%)	*** 38/90 (42.2%)
Alveolar/bronchiolar carcinoma	3/90 (3.3%)	9/90 (10.0%)	4/90 (4.4%)	1/89 (1.1%)	5/90 (5.6%)	3/90 3.3%)
Alveolar/bronchiolar adenoma/carcinoma	25/90 (27.8%)	** 44/90	** 42/90	18/89 (20.2%)	** 36/90	*** 41/90

		(48.9%)	(46.7%)		(40.0%)	(45.6%)
HAEMOLYMPHORETICULAR SYSTEM						
Malignant lymphoma (lymphocytic)	2/90 (2.2%)	0/90 (0%)	1/90 (1.1%)	4/90 (4.4%)	3/90 (3.3%)	2/90 (2.2%)
Malignant lymphoma (mixed)	-	-	-	5/90 (5.6%)	1/90 (1.1%)	* 0/90 (0%)
Histiocytic sarcoma	2/90 (2.2%)	2/90 (2.2%)	0/90 (0%)	5/90 (5.6%)	2/90 (2.2%)	4/90 (4.4%)
UTERUS						
Polyp	-	-	-	3/89 (3.4%)	0/71 (0%)	4/90 (4.4%)
MAMMARY REGION						
Adenocarcinoma	-	-	-	2/90 (2.2%)	5/90 (5.6%)	3/90 (3.3%)
OVARY						
Cystadenoma	-	-	-	3/89 (3.4%)	3/69 (4.3%)	2/90 (2.2%)

Table 2.6.8.1-6: Macroscopic and microscopic findings in the liver (*Aharne1990*); (Incidence/Number of animals); DOS=died on study, SAC=scheduled sacrifice

Dose[ppm]	**0**		**40**		**80**	
	DOS	**SAC**	**DOS**	**SAC**	**DOS**	**SAC**
Chronic inflammation (12-24 months)						
Males	7/29	10/16	26/33	12/12	29/36	1/1
Females	5/24	17/21	17/39	3/4	22/35	4/4
Liver cell hyperthrophy (8-12 months)						
Males	0/2	0/20	0/2	16/20	4/11	19/20
Females	0/1	0/20	0/4	3/20	1/7	4/20
Hepatic necrosis (12-24 months)						
Males	0/29	1/16	11/33	0/12	17/36	0/1
Females	2/24	0/21	5/39	0/4	4/35	1/4
Accentuated liver lobulation (8-12 months)						
Males	0/2	0/20	0/2	11/20	1/11	9/20
Females	0/1	0/20	0/4	1/20	0/7	2/20

Table 2.6.8.1-7: Selected biochemical values; *p<0.05, **p<0.01 *(Anonymous, 1990)*

Dose[ppm]	0		40		80	
	12months	**terminal**	**12months**	**terminal**	**12months**	**terminal**
Alanine aminotransferase [IU/l]						
Males	35	142	127**	267	224	267**
Females	31	41	78**	105**	72*	244
Sorbitol dehydrogenase [IU/l]						
Males	79.6	23.9	148.8**	23.6	139.7**	23.77
Females	69.4	22.0	122.2**	29.7	116.5**	25.8

able 2.6.8.1-8: Survival at study termination (*Anonymous, 1990*)

Dose[ppm]	Males			Females		
	0	**40**	**80**	**0**	**40**	**80**
Survival	15 (17%)	12 (13%)	1 (1%)	21 (23%)	4 (4%)	4 (4%)

Conclusion on UDMH carcinogenicity: The results of chronic toxicity/carcinogenicity studies and 90-day study in mice are in line with the current classification according to CLP criteria (Regulation (EC) No. 1272/200), i.e. UDMH is categorized as a Group 1B carcinogen. As for the mechanism of carcinogenic potential, based on the available data, it cannot by excluded without a doubt that UDMH does not exert intrinsic mutagenic properties.

2.6.8.2 Supplementary studies on the active substance

An immunotoxicity study with daminozide *(Anonymous, 2011)* evaluating anti-sheep red blood cell response in mice, performed according to OPPTS 870.7800 guideline, is available. Daminozide (purity: 100%) at the concentration of 1000, 4000 and 16000 ppm was administered to CD-1 female mice (10 animals/group) in diet for 28 days. On Day 24, a single intravenous dose of 1 x 10^8 sheep red blood cells (SRBCs)/mL was administered to all animals. Five days after the immunisation (Day 29), a serum sample for anti-SRBC IgM titer was collected. Cyclophosphamide, the immunomodulatory positive control, was injected intraperitoneally during days 24 – 28. All animals survived to the scheduled necropsy. There were no treatment-related clinical observations. No effect of the treatment on the body weight, thymus weight, and food consumption was revealed. The water consumption was significantly increased in the top dose at weeks 3 and 4.The absolute and relative spleen weights in 16000 ppm group were significantly higher comparing to the controls, whereas animals treated with cyclophosphamide had absolute and relative spleen weights significantly lower than controls. The slight reductions of comparable magnitude were observed for all three concentrations of daminozide. However, these decreased values were not considered to be toxicologically relevant in the absence of a dose-relationship and statistical significance. On the other hand, cyclophosphamide treatment caused significant reduction in anti-SRBC IgM formation, which is consistent with the immunosuppressive effects of this control article. The NOAEL for immunotoxicity was set at 16000 ppm equal to 2879 mg/kg bw/day.

Table 2.6.8.2-1: Anti-SRBC IgM (U/mL); * = $p<0.05$;

Treatment	Mean	SD
Vehicle	3849.2	530.10
Daminozide 1000 ppm	3219.8	557.93
Daminozide 4000 ppm	2947.8	392.48
Daminozide 16000 ppm	3081.4	476.52
Cyclophosphamide 25 mg/kg bw/day	210.3*	46.33

2.6.8.3 Endocrine disrupting properties

The short-term, long-term, reproduction as well as developmental toxicity studies showed no evidence that daminozide directly interferes with the function of the sexual or thyroid hormone pathways. No effects on fertility, reproduction, development, or sexual maturation were noted. However, taking into account the increased incidence of pituitary adenomas in treated female rats via non-genotoxic mode of action, the potential of daminozide to induce the hormonal imbalance cannot be unequivocally excluded. The mechanistic studies with daminozide or UDMH providing data about selected endocrine mechanism(s) are not available.

2.6.9 Summary of medical data and information

No data available

2.6.10 Toxicological end points for risk assessment (reference values)

Table 55: Overview of relevant studies for derivation of reference values for risk assessment

Species	Study (method/type, length, route of exposure)	Test substance	Critical effect	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	Cross reference
Rat	90-day oral toxicity study Oral route: by gavage	Daminozide Dose levels: 0, 40, 200, 1000 mg/kg bw/day Purity: 100.2%	No adverse effects	1000	-	*Anonymous (2005)*
Dog	1-year oral toxicity study Oral route: in diet	Daminozide Dose levels: 0, 300, 3000, 7500 ppm Purity: 99%	Renal cell adenoma, food-like emesis, soft stool	80.5 (3000 ppm)	199 (7500 ppm)	*Anonymous (1988a)*
Rat	28-day dermal toxicity study	Daminozide 0, 125, 500, 2000 mg/kg bw/day Purity: 100%	No adverse effects	2000	-	*Anonymous (2012)*
Rat	Combined chronic toxicity carcinogenicity study Oral route: in	Daminozide Dose levels: 0, 100, 500, 5000, 10000 ppm	Pituitary adenomas, bile duct hyperplasia	5 (provisional NOAEL)	-	*Anonymous (1988a)*

Species	Study (method/type, length, route of exposure)	Test substance	Critical effect	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	Cross reference
	diet	Purity: 99%				
Mouse	2-year carcinogenicity study Oral route: in diet	Daminozide Dose levels: 0, 300, 3000, 6000 and 10000 ppm Purity: 99%	Pulmonary neoplasms (alveolar/bronchiolar adenomas + carcinomas)	Could not be derived	-	*Anonymous (1988c)*
Rat	Two-generation reproduction toxicity study Oral route: by gavage Duration of exposure: F0: for ten weeks, then throughout mating, gestation, lactation, until sacrifice; F1: in utero, while nursing, then from Day 25 post-partum throughout mating, gestation, lactation, until sacrifice	Daminozide Dose levels: 0, 60, 360 or 1200 mg/kg bw/day Purity: > 99 %	Parental toxicity: loose faeces, perianal fur staining, excessive post-dose salivation Reproductive toxicity: no adverse effects	360 (parental) 1200 (reproductive)	1200 (parental) >1200 (reproductive)	*Anonymous (1994)*
Rat	Two-generation reproduction	Daminozide Dose levels:	Parental toxicity: changes in body	50 (1000 ppm; parental)	500 (10000 ppm; parental)	*Anonymous (1987)*

Species	Study (method/type, length, route of exposure)	Test substance	Critical effect	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	Cross reference
	toxicity study Oral route: in diet Duration of exposure: F0: continuously from approximately 7 weeks of age throughout mating, gestation, lactation, until sacrifice; F1: in utero, while nursing; continuously in the diet after weaning throughout mating, gestation, lactation, until sacrifice	0, 100, 1000 and 10000 ppm Purity: 99%	weight Reproductive toxicity: no adverse effects	500 (10000 ppm; reproductive)	>500 (10000 ppm; reproductive)	
Rat	Prenatal development toxicity study Oral route: by gavage Duration of exposure: once	Daminozide Dose levels: 0, 150, 750 and 1500 mg/kg bw/day Purity: >99%	Parental toxicity: reduced body weight gain Developmental: no adverse effects	150 (parental) 1500 (developmental)	750 >1500	*Anonymous (1993)*

Species	Study (method/type, length, route of exposure)	Test substance	Critical effect	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	Cross reference
	daily between Days 6 and 15 of pregnancy					
Rabbit	Prenatal development toxicity study Oral route: by gavage Duration of exposure: days 7 to 28 of gestation	Daminozide Dose levels: 0, 250, 500 and 1000 mg/kg bw/day Purity: 99.5%	Parental toxicity: mortality, soft/liquid faeces, hyperpnoea, hyperactivity, convulsions Developmental toxicity: the slight reduction in ossification and foetal weight on a litter basis	250 (parental) 500 (developmental)	500 (parental) 1000 (developmental)	*Anonymous (2006a)*
Rabbit	Prenatal development toxicity study Oral route: by gavage Duration of exposure: once daily on days 7 - 19 of gestation	Daminozide Dose levels: 50, 150, and 300 mg/kg bw/day Purity: 99 %	No adverse effects	300 (parental) 300 (developmental)	>300 (parental) >300 (developmental)	*Anonymous (1985)*
Rat	Acute neurotoxicity study Oral route: by gavage; a single dose	Daminozide Dose levels: 0, 500, 1000, 2000 mg/kg bw/day Purity: 100%	Decreased locomotor activity (total distance, basic and fine movement)	1000	2000	*Anonymous (2012a*)

Species	Study (method/type, length, route of exposure)	Test substance	Critical effect	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	Cross reference
Rat	90-day oral neurotoxicity study Oral: by gavage	Daminozide Dose levels: 0, 100, 300, 1000 mg/kg bw/day Purity: 100%	No adverse effects	1000	>1000	*Anonymous (2012b*)
Mouse	28-day immunotoxicity study Oral route: in a diet	Daminozide Dose levels: 0, 1000, 4000, 16000 ppm Purity: 100%	No adverse effects	2879 (16000ppm)	>2879 (16000 ppm)	*Anonymous (2011)*

2.6.10.1 Toxicological end point for assessment of risk following long-term dietary exposure – ADI (acceptable daily intake)

Based on the use pattern of formulations with daminozide, there is no concern for dietary exposure to daminozide (no uses on edible crops). However, since residues of daminozide in drinking water cannot be excluded the acceptable daily intake (ADI) is calculated.

The calculation of the ADI is based on the results of the 2-year carcinogenicity study in rat (*Anonymous, 1988a),* which revealed a provisional NOAEL of 5 mg/kg bw/day. The application of an assessment factor of 100 and additional safety factor of 2 results in ADI of 0.025 mg/kg bw/day.

2.6.10.2 Toxicological end point for assessment of risk following acute dietary exposure - ARfD (acute reference dose)

Not applicable (not necessary).

2.6.10.3 Toxicological end point for assessment of occupational, bystander and residents risks – AOEL (acceptable operator exposure level)

Usually, the AOEL for systemic exposure is set on basis of the lowest NOAEL from short term toxicity studies. However, due to the frequent use pattern of formulations based on daminozide, the provisional NOAEL from 2-year carcinogenicity study in rats being 5 mg/kg bw/day is used for the derivation of the AOEL. By using a safety factor of 100, additional safety factor of 2 and adjustment for 35% oral absorption, this results in a long-term systemic AOEL of 0.009 mg/kg bw/day.

2.6.10.4 Toxicological end point for assessment of occupational, bystander and residents risks – AAOEL (acute acceptable operator exposure level)

Not applicable.

2.6.11 Summary of product exposure and risk assessment

Operator exposure

Both representative products are intended for use in ornamentals with different application rate. For the exposure assessment it was considered the application rate related with outdoor/indoor use. For detail calculation see Volume 3_CP_B6 part B.6.4.1 of individuals products.

Alar

Hand held application - outdoor

Based on the estimations according to UK POEM models a safe use could not be demonstrated for operators applying daminozide with UDMH in the product Alar outdoors by hand held device, even if they use appropriate working clothes and PPE - common workwear , sturdy footwear, protective gloves during mixing/loading and during application and respiratory protection.

Hand held application - indoor

Based on the estimations according to ECPA greenhouse model a safe use could be demonstrated for operators applying daminozide with UDMH in the product Alar indoors by hand held device, if they use appropriate working clothes and PPE – coveralls, sturdy footwear, protective gloves during mixing/loading and during application and respiratory protection. Based on the estimations according to Dutch greenhouse model a safe use could not be demonstrated, even with high level of PPE.

Automated gantry sprayer - indoor

Based on the estimations according to German (BBA) and UK POEM models a safe use could be demonstrated for operators applying daminozide with UDMH in the product Alar indoors by automated gantry sprayer, even without using of PPE.

Dazide Enhance

Hand held application - outdoor

Based on the estimations according to UK POEM models a safe use could not be demonstrated for operators applying daminozide with UDMH in the product Dazide Enhance outdoors by hand held device, even if they use appropriate working clothes and PPE - common workwear , sturdy footwear, protective gloves during mixing/loading and during application and respiratory protection.

Hand held application - indoor

Based on the estimations according to ECPA greenhouse model a safe use could be demonstrated for operators applying daminozide with UDMH in the product Dazide Enhance indoors by hand held device, if they use appropriate working clothes and PPE – coveralls, sturdy footwear, protective gloves during mixing/loading and during application and respiratory protection. Based on the estimations according to Dutch greenhouse model a safe use could not be

demonstrated, even with high level of PPE.

Automated gantry sprayer - indoor

Based on the estimations according to German (BBA) and UK POEM models a safe use could be demonstrated for operators applying daminozide with UDMH in the product Dazide indoors by automated gantry sprayer, even without using of PPE.

Bystander and resident exposure.

Both representative products are intended for use in ornamentals. For the exposure assessment it was considered outdoor application and the application rate related with outdoor use. For the indoor use bystander exposure is not relevant. For detail calculation see Volume 3_CP_B6 part B.6.4.2 of individuals products.

Alar

Based on the estimations according to German model for bystander and resident exposure assessment outdoor applications of daminozide with UDMH in the product Alar are not safe for bystanders – children exposed to daminozide. The estimated value of exposure for bystanders – children is above the AOEL for dermal and inhalation of exposure.

Dazide Enhance

Based on the estimations according to German model for bystander and resident exposure assessment outdoor applications of daminozide with UDMH in the product Dazide are not safe for bystanders – children exposed to daminozide. The estimated value of exposure for bystanders – children is above the AOEL for dermal and inhalation of exposure.

Worker exposure.

Both representative products are intended for use in ornamentals. For the exposure assessment it was considered indoor application and the application rate related with indoor use as the worst case. For detail calculation see Volume 3_CP_B6 part B.6.4.3 of individuals products.

Alar

Based on the estimations according to German model for worker exposure combined with inhalation exposure assessment indoor applications of daminozide with UDMH in the product Alar are not safe for workers, even if they use appropriate working clothes and PPE - common workwear, sturdy footwear, protective gloves during manipulation and respiratory protection. The assessment was refined by DFR and DT50 values obtained from the study provided by applicant, nevertheless estimated values of exposure were still above AOEL.

Dazide Enhance

Based on the estimations according to German model for worker exposure combined with inhalation exposure assessment indoor applications of daminozide with UDMH in the product Dazide are not safe for workers, even if they use appropriate working clothes and PPE - common workwear, sturdy footwear, protective gloves during manipulation and respiratory protection. The assessment was refined by DFR and DT50 values obtained from the study provided by applicant, nevertheless estimated values of exposure were still above AOEL.

2.7 Residue

2.7.1 Summary of storage stability of residues

Not relevant since residue trials are not required to support this use on non-edible crops (ornamentals).

2.7.2 Summary of metabolism, distribution and expression of residues in plants, poultry, lactating ruminants, pigs and fish

Not relevant. Metabolism studies in plants, farm animals or fish are not required since the proposed use is in non-edible crops (ornamentals).

2.7.3 Definition of the residue

Residue definition in plant matrices for risk assessment	Daminozide (sum of daminozide and 1,1-dimethyl-hydrazine (UDMH) expressed as daminozide)
Residue definition in plant matrices for monitoring	Daminozide (sum of daminozide and 1,1-dimethyl-hydrazine (UDMH) expressed as daminozide)
Residue definition in animal matrices for risk assessment	Daminozide (sum of daminozide and 1,1-dimethyl-hydrazine (UDMH) expressed as daminozide)
Residue definition in animal matrices for monitoring	Daminozide (sum of daminozide and 1,1-dimethyl-hydrazine (UDMH) expressed as daminozide)

2.7.4 Summary of residue trials in plants and identification of critical GAP

Not relevant. Residue studies in plants are not required since the proposed use is in non-edible crops (ornamentals).

2.7.5 Summary of feeding studies in poultry, ruminants, pigs and fish

Not relevant. Feeding studies are not required since the proposed use is in non-edible crops (ornamentals).

2.7.6 Summary of effects of processing

Not relevant. Processing studies are not required since the proposed use is on non-edible crops (ornamentals).

2.7.7 Summary of residues in rotational crops

In its review of daminozide MRLs, EFSA noted "that the uptake of daminozide residue in the potential following crops has not been investigated but it is noted that rotation of ornamental crops with edible crops is rather unusual due to their specificities. Moreover, daminozide residues were demonstrated during the peer review to decline rapidly (EC, 2005). Occurrence of residues in edible crops resulting from crop rotation is therefore also not expected." (EFSA Journal 2012;10(4):2650)

Daminozide has a very short DT90 (ca. 2-3 days) and the major metabolite, methanol, was seen at 21% after 16 hours (0.32 mg/kg) reducing to 0.02 mg/kg after 72 hours. A new aerobic soil metabolism study (Möndel, M.; 2014) confirmed these findings; 2 days after treatment, >90% of Daminozide had degraded and 4 days after treatment >80% of the radioactivity was recovered as carbon dioxide. Based on this information, no detectable residues would be

expected in following crops.

2.7.8 Summary of other studies

No other studies conducted/required.

2.7.9 Estimation of the potential and actual exposure through diet and other sources

Not relevant since the proposed use is in non-edible crops (ornamentals).

Nevertheles a theoretical maximum daily intake (TMDI) calculation has been carried out using the EFSA PRIMo model version 2, the current ADI for daminozide (0.45 mg/kg bw/day) and the MRLs for Daminozide). The highest TMDIs were calculated as 1.0% for UK Infant, 0.9% for UK Toddler, FR Toddler, NL child.

Acute exposure calculations were not carried out because an ARfD was not deemed necessary.

2.7.10 Proposed MRLs and compliance with existing MRLs

Following the review under Article 12(1) of Regulation (EC) 396/2005 EU MRLs were adopted in Commission Regulation (EU) No 2017/624 of 30 March 2017. MRLs are all set at the limit of quantification which ranges between 0.06*-0.1* mg/kg for crops, 0.06* mg/kg for products of animal origin and for honey.

There are no proposals to amend any of these MRLs on the basis of the supported use on ornamentals.

2.7.11 Proposed import tolerances and compliance with existing import tolerances

Daminozide had previously set MRLs under the Codex process. However, these have subsequently been withdrawn by the Codex Alimentarius Commission as the edible uses themselves were withdrawn by registration holders.

2.8 Fate and behaviour in the environment

2.8.1 Summary of fate and behaviour in soil

The route of aerobic soil degradation of [^{14}C]-daminozide was investigated in four soils incubated in the laboratory in the dark at 20ºC, and with a soil moisture content of 40% MWHC, in the study of Möndel, 2015. [^{14}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^3). Daminozide degraded rapidly in all four soils to <0.1% of applied radioactivity in all soils after 7 days incubation. 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 (a single replicate in Soil III is excluded due to losses of $^{14}CO_2$). Organic volatile compounds were always <0.1% AR. Unextracted residues 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 1-2 days after application. Concentrations then declined rapidly such that final concentrations in all soils were ≤ 2.4% AR 34 DAT. No other fraction exceeded 0.3% AR.

The original assessment of daminozide presented in Volume 3, Section B.8 of the DAR (the Netherlands 1999) was based upon several studies. In total the aerobic degradation of daminozide was studied in 10 soils, 6 of which were considered acceptable. The 6 soils displayed an appropriate range of textural classes, organic carbon contents (0.41 – 4.25% OC), and soil pH (4.1 – 7.2). The previous assessment concluded that in soil aerobic degradation studies the

primary degradation product was CO_2 (20-59% AR after 2 – 64 days) with bound residues being formed at concentrations of 20 to 25% AR after 2-3 days. Formaldehyde was reported to have been observed at concentrations up to 21% AR in the study of Yu and Kobryn, 1993. Other minor metabolites were detected, but never exceeded 5% AR and do not correspond to UDMH, NDMA, dimethlyhydrosamine or dimethylhydrazine.

All studies assessed in the DAR show some deficiencies. Consequently, these studies are considered as supporting information only. Definitive end-points for the aerobic soil degradation are considered to be derived from the study of Möndel, 2015 only.

Considerable efforts were made to identify the unknown polar metabolite, M1, observed in the study of Möndel, 2015. Multiple HPLC and LC/MS techniques were explored, either directly or after derivatisation in the studies of Möndel, 2015 and Jones, 2015. These attempts were not successful, and a definitive conclusion on the identity of the metabolite M1 was not possible. However, the investigations demonstrated that the metabolite does not correspond to the available reference items dimethylamine, NDMA, UDMH, and a derivatisation technique demonstrated that the metabolite is not any other hydrazine. A derivatisation technique with 2,4-dinitrophenylhydrazine (DNPH) and analyses with LC/MS neither excluded nor confirmed that M1 is formaldehyde. Other HPLC analyses showed that M1 might also be methanol. The metabolite was observed to be polar, volatile, and highly soluble in water.

A further attempt to characterise the metabolite M1 in the soil extracts was made in the study of DeMaio, 2015. Investigations within the study confirmed that the polar metabolite was not UDMH, dimethylamine or NDMA. The derivatisation technique with DNPH was repeated to determine whether the polar metabolite was formaldehyde. A positive identification of the metabolite, M1, as formaldehyde was not able to be made. Further work was performed with ion exclusion HPLC with LC-Refractive Index (RI) detection. The unknown radioactive peak was retained and formaldehyde and formic acid were excluded on the basis of their reference standard's retention times in the analysis. However, M1 was identified as methanol by comparison to the reference standard, co-chromatography with methanol fortified extracts and refractive index and radioactivity flow detection in series. The RMS agrees that it is likely that unknown metabolite M1 is methanol but 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.

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

In the original review, a polar fraction in the study of Yu and Kobryn, 1993, which displayed similar maximum formations and degradation rates to those for M1 was identified as formaldehyde. 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.

As discussed, the new study of Möndel, 2015, 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 identified 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.

The anaerobic route of degradation of [^{14}C]-daminozide in soil was investigated in the study of Dzialo and Harned, 1986, which was assessed in Volume 3, Section B.8 of the DAR for daminozide (the Netherlands 1999). Low recoveries were observed in some samples, which were considered to be due to formation of methane and ethane, and due to the loss of CO_2 during the analysis procedure. Due to several deficiencies the study was not considered acceptable. Anaerobic soil conditions will not 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, the anaerobic degradation of daminozide is not a significant route of degradation and does not require further consideration.

UV/visible absorption spectra for daminozide summarised in Volume 3, Section B-2 DAR for daminozide, and the new spectra from the study of Kelly, 2011, displayed no or negligible absorption of light at wavelengths >290 nm. Therefore, daminozide would not be expected to undergo photolytic degradation in soil.

Overall, following application, daminozide is expected to undergo very rapid aerobic degradation in soil to the terminal metabolite CO_2, via methanol (maximum formation: 27.2% AR). Anaerobic conditions are not expected to be encountered because of the proposed use and daminozide's very rapid degradation under aerobic conditions, while the photolytic degradation in soil of daminozide is anticipated to be negligible.

The study of Möndel, 2015, is considered to provide the definitive end-points for the rate of degradation of daminozide in laboratory aerobic soil degradation studies. SFO DT_{50} values of 0.1 – 0.4 days were calculated in all four soils for the aerobic degradation of daminozide in the dark at 20ºC 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_{50} for daminozide, corrected to 20ºC and pF2 for use in modelling, of 0.12 days was calculated.

Table 2.8.1-1: Summary of the calculated DT50 values for daminozide in aerobic soil degradation studies

Soil, USDA classification	Kinetic model	pH (0.01M $CaCl_2$)	Temp. (ºC)	Soil Moisture (g/100g)	Water holding capacity at pF2 (g/100g)	Correction Factor	DT_{50}/ DT_{90} (days)	χ^2 error (%)	Corrected DT_{50} – 20ºC & 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
Geometric Mean									**0.12**

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

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 dissipation data are not required for daminozide because of its very rapid degradation rate. Nevertheless, a single field study conducted at a site in Connecticut, USA. Study was not considered acceptable by the RMS.

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 20ºC, and following correction to a soil moisture content of pF2 is presented in Table below. A geometric mean DT_{50} corrected to 20ºC and pF2 for use in modelling, of 3.9 days was calculated. A mean formation fraction of 0.27 was also calculated.

Table 2.8.1-2: Summary of the calculated DT50 values for the metabolite M1 following application of daminozide in aerobic soil degradation studies

Soil, USDA classification	pH (0.01M $CaCl_2$)	Temp. (ºC)	Soil Moisture (g/100g)	Water holding capacity at pF2 (g/100g)	Correction Factor	DT_{50}/ DT_{90} (days)	Formation Fraction	χ^2 error (%)	Corrected DT_{50} – 20ºC & 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
Fislis – Silt loam	6.8	20	12.8*	42.3	0.43	4.5/ 15.0	0.29	18.3	1.9
Geometric Mean							-	-	**3.9**
Arithmetic Mean							**0.27**	-	**4.2**

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

Adsorption and mobility in soil

The batch adsorption/desorption study of Spare (1987) was performed on four soils for daminozide. Kfoc values were 18.4 – 46.5 mL/g, and 1/n values were 1.11 – 1.37. Arithmetic mean values from the four soils were 26.6 mL/g and 1.27. A summary of individual soil adsorption parameters is presented in Table 2.8.1-3. Due to several deviations of the study, new adsorption/desorption study is required.

Table 2.8.1-3: Adsorption Kf, Kfoc and 1/n (Freundlich exponent) values for daminozide

Soil Selection	Soil pH	K_f [mL/g]	K_{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

Batch adsorption studies were not performed for methanol because of the practical difficulties created by its high volatility, difficulties in robust analysis and high 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 $LogK_{ow}$ method and 2.75 L/kg from the experimental database were obtained.

In the aged soil column leaching study of McManus *et al*. (1984) 56% of the applied radioactivity was observed in leachate, 84.3% of which was characterised as daminozide. All other radioactive fractions were < 5% AR, indicating that the occurrence in groundwater of soil metabolites of daminozide will be low. Analysis of radioactivity remaining in the soil column displayed only polar products.

2.8.2 Summary of fate and behaviour in water and sediment [equivalent to section 11.1 of the CLH report template]

Daminozide was not hydrolysed in aqueous buffer solutions at pH 5 – 9. Daminozide is stable to aqueous photolysis in sterilised irradiated pure water, but the aqueous photolysis study of Brice and Scholey (2006), demonstrated that daminozide can be photolytically degraded in irradiated sterilised natural waters. However, the rate of degradation (DT_{50} = 36.8 days) was very slow compared to the degradation observed in water/sediment systems. The stability of daminozide to photolysis in pure waters, and the slow photolytic degradation in natural waters, is supported by the results of the UV/vis spectra, which indicate that daminozide does not absorb light with wavelengths >290 nm to a significant degree.

Daminozide was shown to be readily biodegradable in the study of Ritter, 1989a, assessed in the original DAR evaluation. The aerobic mineralisation in surface water study of Button, 2015, further demonstrated that daminozide is rapidly biodegraded. In the study, conducted in the dark at 20ºC in surface water system at nominal [^{14}C]-daminozide application rates of 2 µg/L and 10 µg/L. Due to several deviations, the enpoints from this study are not considered valid. Daminozide degraded via an unknown polar component (likely to be methanol) with mean maximum concentrations of 35.4 - 75.7% AR, 2 - 3 days after treatment.

The rapid biotic degradation of daminozide in water/sediment systems was demonstrated in the study of De Vette and van Es (2002). Whole system DT_{50} values of 0.88 days and 0.94 days were calculated for daminozide in accordance with FOCUS Kinetics guidance. 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. Evolved $^{14}CO_2$ increased up to a maximum of 38.9% AR 7 days after application, whereupon a plateau was reached.

Re-examination of the study report of De Vette and van Es, 2002, demonstrates that the study only utilised HPLC analysis with radio- and UV detection. No confirmatory analytical method was reported and the only reference standard for potential polar metabolites investigated was that for formaldehyde. Therefore, though the formaldehyde standard eluted with a comparable retention time to the metabolite peak in the 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. The identification of the polar metabolite as formaldehyde within the study is therefore unreliable. Later it was confirmed that the unknown metabolite can only be methanol.

The study demonstrates that in natural water/sediment systems daminozide is likely to rapidly degrade to a polar metabolite (considered most likely to be methanol) which in turn is degraded to CO_2. For the polar metabolite, whole system DT_{50} values of 37.8 days and 93.4 days, with formation fractions of 0.283 and 0.258 were calculated.

Overall, the dominant route of degradation in natural aquatic systems is likely to be biotic, with daminozide degrading rapidly to methanol and then to CO_2. Photolytic degradation is unlikely to be significant.

2.8.2.1 Rapid degradability of organic substances

Table 56: Summary of relevant information on rapid degradability

Method	Results*	Key or Supportive study[1]	Remarks	Reference
Daminozide: Aerobic mineralisation in surface water, OECD 309	Results are not considered reliable	Not acceptable	New study is required	Button, S. (2015)
A study on the degradation of [^{14}C] daminozide in two water/sediment systems, OECD 308 (draft)	DT50 = 0.878 – 0.935 days in whole system for daminozide	Key	-	De Vette, H.Q.M., van Es, C. (2002)

** data on full mineralization should be reported*

Assessment in relation to the P-criteria

Following criteria for persistence in water and sediment are stated in Annex II to Regulation (EC) 1107/2009:

- DT50 in water: POP – 60 days, PBT – 40 days (fresh) and 60 days (marine), vPvB – 60 days (all water)

- DT50 in sediment: POP – 180 days, PBT – 120 days (fresh) and 180 days (marine), vPvB – 180 days (all sediment)

Normalised laboratory soil DT50 to 12°C = 0.1 – 0.5 days

Normalised whole system water/sediment DT50 to 12°C = 1.9 – 2.0 days

No reliable DT50 in surface water, data gap

Adsorption to sediments is minimal with levels not being observed above 6.7% of AR for daminozide.

Therefore, available study results for daminozide are below P-criteria.

2.8.2.1.1 Ready biodegradability

Table 57 : Summary of relevant information on ready degradability

Method	Results	Remarks	Reference
Test type: ready biodegradability activated sludge, domestic (adaptation not specified) OECD Guideline 301 E (Ready biodegradability: Modified OECD Screening Test)	readily biodegradable % Degradation of test substance: 82 after 28 d The 10-day window was met	1 (reliable without restriction) key study experimental result **Test material (EC name): daminozide**	Ritter, A. (1989)

2.8.2.1.2 BOD5/COD

Data not available

2.8.2.2 Other convincing scientific evidence

Data not available

2.8.2.2.1 Aquatic simulation tests

Data not available

2.8.2.2.2 Field investigations and monitoring data (if relevant for C&L)

Data not available

2.8.2.2.3 Inherent and enhanced ready biodegradability tests

Data not available

2.8.2.2.4 Soil and sediment degradation data

Data on soil degradation are reported under 2.8.1, whilst sediment degradation data are presented under 2.8.2

2.8.2.2.5 Hydrolysis

Data on hydrolysis are reported under 2.8.2.

2.8.2.2.6 Photochemical degradation

Data on photochemical degradation are reported under 2.8.1 and 2.8.2.

2.8.2.2.7 Other / Weight of evidence

None

2.8.3 Summary of fate and behaviour in air

The vapour pressure and Henry's Law Constant for daminozide are 1.5 x 10^{-6} Pa at 25ºC and 1 x 10^{-9} Pa m^3/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 x 10^6 cm^{-3}. 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.

Methanol is known to be volatile, and a vapour pressure of 1.69 x 10^4 Pa at 25ºC and a Henry's Law constant of 0.46 Pa.m^3/mole at 25ºC 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).

2.8.3.1 Hazardous to the ozone layer

Data not available

2.8.3.1.1 Short summary and overall relevance of the provided information on hazards to the ozone layer

Considering the vapour pressure and Henry's Law constant values reported above, daminozide is not anticipated to be volatilised to air. The calculated Atkinson half-life demonstrates that any daminozide that is volatilised would be rapidly degraded. Daminozide is not anticipated to be subject to long range transport.

Methanol is known to be volatile with a vapour pressure of 1.69 x 10^4 Pa at 25ºC and a Henry's Law Constant of 0.46 Pa.m^3/mole at 25ºC 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). 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.

2.8.3.1.2 Comparison with the CLP criteria

The substance is not mentioned in Annexes of the Montreal Protocol.

2.8.3.1.3 Conclusion on classification and labelling for hazardous to the ozone layer

Not classified

RAC evaluation of hazards to the ozone layer

Summary of the Dossier Submitter's proposal

The vapour pressure (1.5 x 10^{-6} Pa at 25 ºC) and Henry's Law Constant (1 x 10^{-9} Pa m^3/mole) for daminozide indicate low volatility. 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 x 10^6 cm^{-3}. A half-life in the upper atmosphere of 10.57 hours or 0.88 days (based on a 12 hour day) was calculated. Based on the available data, daminozide is not anticipated to be volatilised to air. Any daminozide that is volatilised would be anticipated to be rapidly degraded. Consequently, daminozide is not anticipated to be subject to long-range transport. No other relevant data has been submitted by the Dossier Submitter.

Comments received during public consultation

Two comments were received, from an MSCA and company-manufacturer. Both agreed with DS proposal not to classify the substance as hazardous to the ozone layer.

Assessment and comparison with the classification criteria

Transport of daminozide in air is considered to be negligible due to its very low vapour pressure and Henry's constant, whilst its photochemical oxidative degradation in air is expected to be rapid. Therefore, local and global effects are expected to be negligible.

Thus, RAC agrees with the DS proposal that **no classification is warranted for this hazard class.**

2.8.4 Summary of monitoring data concerning fate and behaviour of the active substance, metabolites, degradation and reaction products

No monitoring data are available for daminozide.

2.8.5 Definition of the residues in the environment requiring further assessment

Soil: daminozide, methanol

Surface water: daminozide, methanol

Sediment: daminozide, methanol

Ground water: daminozide, methanol

Air: daminozide, methanol

2.8.6 Summary of exposure calculations and product assessment

Soil:

PECsoil values for daminozide and its polar metabolite M1 (methanol) in soil have been calculated for the proposed GAP uses of daminozide as a plant growth regulator on ornamental crops. PEC values were calculated for applications made in the field and also indoor (calculated by the RMS), at a maximum application rate of 5 x 4.25 kg a.s./ha (field) and 5 x 7.65 kg a.s./ha (indoor), with a minimum application interval of 7 days. Calculations were performed according to FOCUS 1997, based on a standard dry soil bulk density of 1.5 g/cm^3 and a soil mixing depth of 5 cm.

The PEC values are presented together with the corresponding TER in section 2.9.9. Complete calculation is presented in Volume 3 (PPP), B-8, B.8.2.

Groundwater:

The fate and behaviour of daminozide and its soil metabolite methanol in groundwater was investigated using the FOCUS groundwater scenarios and the FOCUS PEARL 4.4.4 model. PECs in groundwater were calculated for both compounds for applications of daminozide made in accordance with the proposed indoor (5 x 7.65 kg a.s./ha; 7 day application interval) and field (5 x 4.25 kg a.s./ha; 7 day application interval) GAPs. In accordance with the GAPs, applications may be made to actively growing plants, and are therefore typically made from spring to late summer. Leaching through the soil profile is typically higher in spring than summer and therefore modelling was performed for applications made in spring to address the worst case situation. Consequently, the first application was assumed to be made on 1st April.

Indoor scenarios are not yet available and therefore for applications to indoor crops the outdoor FOCUS scenarios were considered. This is likely to represent a significant over-estimation of groundwater concentrations for glasshouse uses, since irrigation water volumes are likely to be much lower than the water volumes experienced as a result of precipitation events which would result in the high and/or prolonged soil moisture contents that result in significant leaching through the soil profile.

Because methanol has a high vapour pressure (1.69 x 10^4 Pa at 25ºC), which is expected to result in losses of the metabolite from soil via volatilisation, only modelling with FOCUS PEARL 4.4.4 was performed. FOCUS PELMO does not consider volatilisation of metabolites.

PECgw values for daminozide were <<0.1 µg/L in all scenarios, for applications made both in the field and in

glasshouses. For methanol, applications made both indoors and in the field resulted in all scenarios displaying PECgw values <0.1 µg/L. A relevance assessment for methanol is therefore not required. Reported PECgw values were the 80th percentile annual average concentrations from 20 years. PECgw values from modelling with FOCUS PEARL 4.4.4 for daminozide and methanol are shown in Table 2.8.6-1 – Table 2.8.6-2.

Table 2.8.6-1: PECgw (µg/L) values for daminozide and its metabolite, methanol, after application to ornamental crops grown indoors

LOCATION	Daminozide (µg/l)	Methanol (µg/l)
CHATEAUDUN	<0.0001	0.004
HAMBURG	<0.0001	0.028
KREMSMUENSTER	<0.0001	0.048
OKEHAMPTON	<0.0001	0.087
PIACENZA	<0.0001	0.046
PORTO	<0.0001	0.023
SEVILLA	<0.0001	<0.0001
THIVA	<0.0001	<0.0001

Table 2.8.6-2: PECgw (µg/L) values for daminozide and its metabolite, methanol, after application to ornamental crops grown in the field

LOCATION	Daminozide (µg/l)	Methanol (µg/l)
CHATEAUDUN	<0.0001	0.002
HAMBURG	<0.0001	0.016
KREMSMUENSTER	<0.0001	0.027
OKEHAMPTON	<0.0001	0.048
PIACENZA	<0.0001	0.026
PORTO	<0.0001	0.013
SEVILLA	<0.0001	<0.0001
THIVA	<0.0001	<0.0001

Surface water and sediment:

Predicted environmental concentrations in surface water (PECsw) and sediment (PECsed) for daminozide and its metabolite methanol were calculated.

Calculations/ modelling of daminozide and methanol PEC values were undertaken based on the GAPs on ornamental crops in the EU. This covered an application of 5 x 7.65 kg a.s./ha (7 day interval) for applications made indoors, and 5 x 4.25 kg a.s./ha (7 day interval) for applications made in the field. Applications of daminozide are to be made to ornamental crops, both <50 cm and >50 cm, both in glasshouses and in the field.

For indoor applications, calculations were performed outside the FOCUS models, based upon a worst case loss of active substance of 0.1% of applied, which was assumed in accordance with existing Dutch guidance.

For field applications calculations/simulations were performed for both daminozide at FOCUS Steps 1-3 and fro methanol at FOCUS Steps 1-2, according to the FOCUS surface water guidance document. In this case Step 4

assessments were not required as an acceptable risk was demonstrated in ecotoxicological risk assessments.

The PEC values are presented together with the corresponding TER in section 2.9.9. Complete calculation is presented in Volume 3 (PPP), B-8, B.8.5.

Air:

Short range transport: Daminozide has a low volatility; its vapour pressure value and Henry's Law Constant are 1.5 x 10^{-6} Pa at 25ºC and 1 x 10-9 Pa m^3/mole respectively. Methanol has very high vapour pressure and therefore the short-range transport is required to be considered.

Long range transport: The calculated Atkinson half-lives of 10.570 hours and 17.4 days for daminozide and methanol respectively, demonstrate that any daminozide that are volatilised would be rapidly degraded. However, the long half-life of methanol indicates that it may be a subject for long-range transport. No data have been submitted by the Notifier.

Other Routes of exposure:

None.

2.9 Effects on non-target species

2.9.1 Summary of effects on birds and other terrestrial vertebrates

Effects on birds

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

Table 58:Summary of avian toxicity studies for daminozide

Test species	Test substance	Test system	Endpoint	Toxicity (mg/kg bw/day)	Reference
Bobwhite quail (*Colinus virginianus*) [# 2]	Daminozide	Acute, oral 14 d	LD_{50}	>2250 mg/kg bw* **>4248 mg/kg bw[3]**	*Anonymous (2006)*
Mallard duck (*Anas platyrhynchos*) [#]	Daminozide	Acute, oral 14 d	LD_{50}	>2250 mg/kg bw* **>4248 mg/kg bw[3]**	*Anonymous (1992)*
Bobwhite quail (*Colinus virginianus*) [# 1]	Daminozide	Short-term dietary, 5 day feeding	LC_{50} LDD_{50}	n.a.	*Anonymous (1977)*
Mallard duck (*Anas platyrhynchos*) [# 1]	Daminozide	Short-term dietary, 5 day feeding	LC_{50} LDD_{50}	n.a.	*Anonymous (1974)*
Bobwhite quail (*Colinus virginianus*) [# 1]	Alar 85	Short-term dietary, 5 day feeding	LC_{50} LDD_{50}	n.a.	*Anonymous (1966a)*
Bobwhite quail (*Colinus virginianus*)	Daminozide	Subchronic and reproductive, 21 weeks feeding	NOEC NOEL	1000 ppm* **79.7 mg/kg bw/d***	*Anonymous (2012)*

[#] Study evaluated in old DAR (1999).
[*] Maximum dose tested.

[1] Study is not considered valid
[2] A limit test.
[3] Extrapolated from the reported endpoint of >2250 mg a.s./kg bw, based on a factor of 1.888
Endpoints used in the regulatory risk assessment included in bold.

Since the acute oral toxicity study with bobwhite quail is a limit test and no mortality was observed at a limit dose >2250 mg/kg, which tested 10 individuals, an extrapolation factor of 1.888 can be applied to the acute endpoints of >2250 mg a.s./kg bw in accordance with the EFSA Guidance on risk assessment for birds and mammals (2009), resulting in LD_{50} value of **4248 mg a.s./kg bw** for birds.

Regarding the other acute toxicity study carried out with mallard duck, similarly no mortality and no effects on body weight and food consumption were observed at any dose tested, including the highest dose of 2250 mg/kg.

Therefore, the extrapolation factor of 1.888 can also be applied to this acute endpoint and it is justified to use the extraplated LD_{50} value of **4248 mg a.s./kg bw** in acute risk assessment for birds.

Effects on terrestrial vertebrates other than birds

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

Table 59: Summary of mammalian toxicity studies for daminozide

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

		0, 60, 360 and 1200 mg/kg bw/day		Developmental: 1200 mg/kg bw/d Fertility: 1200 mg/kg bw/d	
Daminozide	Rat	Developmental (gavage), OECD 414, 0, 150, 750 and 1500 mg/kg bw/day	NOEL	Maternal: 150 mg/kg bw/d body weight gain, food consumption Developmental: 1500 mg/kg bw/d Teratogenicity: 1500 mg/kg bw/d	*Anonymous, 1993*
Daminozide [1]	Rat	Developmental (in diet), 0, 300, 600 and 1000 mg/kg bw/day	NOEL	Maternal: 1000 mg/kg bw/d Developmental: 1000 mg/kg bw/d Teratogenicity: 1000 mg/kg bw/d	Khera et al., 1979
Daminozide	Rabbit	Developmental (gavage), OECD 414 0, 50 150 and 300 mg/kg bw/day	NOEL	Maternal: 300 mg/kg bw/d Developmental: 300 mg/kg bw/d Teratogenicity: 300 mg/kg bw/d	*Anonymous., 1985*
Daminozide	Rabbit	Developmental (gavage), OECD 414, 0, 300, 500 and 700 mg/kg bw/day	NOEL	Maternal: 250 mg/kg bw/d clinical signs and mortality Developmental: **500 mg/kg bw/d** slight reduction in ossification and litter weight. Teratogenicity: 1000 mg/kg bw/d	*Anonymous, 2006b*

[1] Study considered as supplementary only.
Endpoints in bold have been considered in the risk assessment

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

The Notifier suggested to use a developmental NOEL of 1200 mg a.s./kg bw/d from the 2-generation rat study by Anonymous (1987), for Notifier´s justification and RMS comment see Volume 3 CP B.9.

Based on the data provided in Volume 3 CP B.9, RMS proposes **NOAEL of 500 mg/kg bw/d as the long-term ecotoxicologically relevant endpoint for wild mammals** derived from the developmental rabbit study by Anonymous (2006b).

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

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

2.9.2 Summary of effects on aquatic organisms [section 11.5 of the CLH report]

2.9.2.1 Bioaccumulation [equivalent to section 11.4 of the CLH report template]

Table 60: Summary of relevant information on bioaccumulation

Method	Species	Results	Key or Supportive study[1]	Remarks	Reference
Not applicable	Not applicable	No experimental data are available.	Not applicable	Not applicable	Not applicable

2.9.2.1.1 Estimated bioaccumulation

The experimentally derived log Kow of daminozide is -1.53 at 20°C (pH 7). For classification and labelling purposes, a substance with Log Kow <4 may be considered unlikely to bioaccumulate in aquatic organisms. Therefore, daminozide has a low potential for bioaccumulation.

2.9.2.1.2 Measured partition coefficient and bioaccumulation test data

For pesticide registration, a Log Kow >3 triggers the requirement for a bioconcentration study. Since the log Kow of daminozide is <3 a bioconcentration study was not conducted and not required.

2.9.2.2 Acute aquatic hazard [equivalent to section 11.5 of the CLH report template]

Table 61: Summary of relevant information on acute aquatic toxicity

Method	Species	Test material	Results	Key or Supportive study	Remarks	Reference
OECD 203 (1992)	Common carp (*Cyprinus carpio*)	Dazide Enhance SG	96 h LC_{50} 420 mg form./L	Acceptable Key study	-	*Anonymous (2009);*

			357 mg a.s./L (mortality) (nom)			
OECD 203 (1992)	Common carp (*Cyprinus carpio*)	Dazide Enhance	96 h LC_{50} 75.5 mg/L (mortality) (mm)	Acceptable Key study	-	*Anonymous (2010);*
US EPA 72-2 (1975)	*Daphnia magna*	Daminozide	96 h EC_{50} 75.5 mg/L (immobility) (mm)	Acceptable Key study	-	Lintott (1992); A.7.4.1.8
OECD 201 (1984)	Freshwater green (*Pseudokirchneriella subcapitata*)	Daminozide	72 h E_rC_{50} >100 mg/L 72 h E_bC_{50} >100 mg/L (growth inhibition) (nom)	Acceptable Key study	-	Manson & Scholey (2006); 2242/049-D2149
OECD 201 (2011)	Freshwater cyanobacteria (*Anabaena flos-aquae*)	Daminozide	72 h E_rC_{50} >100 mg/L 72 h EyC_{50} >100 mg/L (growth inhibition) (nom)	Acceptable Key study	-	Seeland-Fremer & Mosch (2014); 87711210

2.9.2.2.1 Acute (short-term) toxicity to fish

No valid study performed with active substance daminozide was available. No measurements of actual concentration of the test substance had been carried out in all three studies (*Anonymous 1972, Anonymous 1977, Anonymous 1987*) and due to unclear exposure during the test, no reliable endpoint could be derived from any of them.

Two valid studies performed with formulations were available, both on *Cyprinus carpio*. They were used for both risk assessment and classification purposes:

Anonymous (2009): Fish (common carp (*Cyprinus carpio*)) were exposed, in groups of seven, to an aqueous solution of the test item (Dazide Enhance SG; 84.9% w/w daminozide) over a range of concentrations of 0, 100, 180, 320, 560 and 1000 mg/L for a period of 96 hours under semi-static conditions. The number of mortalities and any sub-lethal effects of exposure in each vessel were determined 3 and 6 hours after the start of exposure and then daily throughout the test until termination after 96 hours.

The measured test item concentrations ranged from 80% – 101% of nominal. Therefore, the effect parameters are expressed in terms of analytically confirmed nominal concentrations.

The 96h-LC_{50} of the test item to common carp based on nominal test concentrations was 420 mg formulation/L (equivalent to 357 mg daminozide/L).

Anonymous (2010): Fish (common carp (*Cyprinus carpio*)) were exposed, in groups of seven, to an aqueous solution of the test item (Dazide Enhance; 85.5% w/w daminozide) over a range of concentrations of 0,10, 18, 32, 56 and 100

mg/L for a period of 96 hours under semi-static conditions. The number of mortalities and any sub-lethal effects of exposure in each vessel were determined 3 and 6 hours after the start of exposure and then daily throughout the test until termination after 96 hours.

The measured test item concentrations ranged from 90% – 104% of nominal. Therefore, the effect parameters are expressed in terms of analytically confirmed nominal concentrations.

The 96h-LC_{50} of the test item to common carp based on nominal test concentrations was 75 mg formulation/L (equivalent to 64 mg daminozide/L).

2.9.2.2.2 Acute (short-term) toxicity to aquatic invertebrates

One valid study performed with active substance daminozide was available:

Lintott (1992): The acute toxicity of the test material to *Daphnia magna* was investigated in a study conducted in accordance with the standardised guideline OECD 202. A 96 hour toxicity test with *D. magna* was conducted using six test concentrations of the test material; 7.8 to100 mg/L, plus control, under flow-through conditions (flow rate 2 mL/min). Ten daphnids < 24 hour old per vessel were tested, two vessels per concentration were used. Actual concentrations were measured by LC at initiation and termination. Actual concentrations ranged from 101 to 112% of nominal. Water temperature ranged between 19 to 21ºC. pH of dilution water ranged from 5.1 to 7.7. Statistics was based on the binomial method. Based on mean measured concentrations, the 96 hour EC50 was 75.5 mg/L (95% confidence interval 66.2 to 101 mg/L).

Two other studies were considered invalid (Leblanc 1976, Abram 1987) since no measurements of actual concentration of the test substance had been carried out and due to unclear exposure during the test, no reliable endpoint could be derived.

2.9.2.2.3 Acute (short-term) toxicity to algae or aquatic plants

Two valid studies performed with active substance daminozide were available:

Manson and Scholey (2006): The effects of the test material on the growth of green algae, *Pseudokirchneriella subcapitata*, were determined in a static system. The study was performed in accordance with the standardised guideline OECD 201. Three replicate algal suspensions were each exposed to nominal concentrations of 4.27, 9.39, 20.7, 45.5 and 100 mg a.s./L for 72 hours. Six replicates without test item were used as controls. Observations of cell growth were recorded at 24, 48 and 72 hours to determine the potential effect on area under growth curve and growth rate. The measured concentrations of the test material were in the range of 92 to 102% of the nominal values. All study results were therefore based on nominal concentrations. Under the experimental conditions, the 72 hour EC50 for both areas under growth curve and growth rate of the test material for *P. subcapitata* was higher than 100 mg a.s./L. The NOEC was 100 mg a.s./L for both endpoints.

Seeland-Fremer and Mosch (2014): The effects of daminozide on the growth of the freshwater green algae *Anabaena*

flos-aquae were determined in a static system. Three replicate algal suspensions were each exposed to nominal concentrations of 0.317, 1.00, 3.16, 10.0, 31.6 and 100 mg a.s./L for 72 hours. Six replicates without test item were used as control. Observations of cell growth were recorded at 24, 48 and 72 hours to determine the potential effect on algal growth rate and yield, relative to the control. The measured concentrations of the test item daminozide at the start of the exposure (0 hours) were in the range of 90 to 101% of the nominal values. The measured concentrations of the test item at the end of the exposure (72 hours) were in the range of 99 to 107% of the nominal value. All study results are therefore based on nominal concentrations. Under the experimental conditions, the 72-hour ErC_{50} and the 72-hour EyC_{50} of daminozide for *Anabaena flos-aquae* were both higher than 100 mg a.s./L. The NOEC was 100 mg a.s./L for growth rate and for yield.

Two other studies were considered invalid (Douglas & Pell 1986, Abram 1987) since no measurements of actual concentration of the test substance had been carried out and due to unclear exposure during the test, no reliable endpoint could be derived.

2.9.2.2.4 Acute (short-term) toxicity to other aquatic organisms

One study on *Lemna* carried out with active substance daminozide was available (Palmer et al. 2001). This study is not considered suitable for regulatory use since percentage inhibition of growth rate was not calculated and biomass was only observed on day 7, therefore, no estimate of starting biomass was available.

2.9.2.3 Long-term aquatic hazard [equivalent to section 11.6 of the CLH report template]

Table 62: Summary of relevant information on chronic aquatic toxicity

Method	Species	Test material	Results	Relevant study	Remarks	Reference
OECD 210 (1992), U.S. EPA OPPTS 850.1400	Fathead minnow (*Pimephales promelas*)	Daminozide	21 d NOEC 1.7 mg/L (growth, mortality) (mm)	Acceptable Key study	-	Anonymous. (2014);
OECD 201 (1984)	Freshwater green (*Pseudokirchneriella subcapitata*)	Daminozide	72 h NOEC 100 mg/L (growth inhibition) (nom)	Acceptable Key study	-	Manson & Scholey (2006); 2242/049-D2149
OECD 201 (2011)	Freshwater cyanobacteria (*Anabaena flos-aquae*)	Daminozide	72 h NOEC 100 mg/L (growth inhibition) (nom)	Acceptable Key study	-	Seeland-Fremer & Mosch (2014); 87711210

2.9.2.3.1 Chronic toxicity to fish

One study with active substance daminozide was available and was considered acceptable:

Anonymous (2014): The objective of this study was to determine the effects of daminozide on the time to hatch, hatching success, survival and growth of fathead minnow during early life-stage development. The study was conducted under flow through conditions for 33 days (a 5-day hatching period plus a 28 -day post-hatch growth period). The

nominal test concentrations were 0, 0.26, 0.64, 1.6, 4.0 and 10 mg a. s. /L. Observations were made at least daily to determine hatching rates and the number of mortalities and signs of toxicity in each treatment group. The mean measured concentrations ranged from 97.8 to 103% of nominal concentrations, nonetheless the results were expressed in terms of mean measured concentrations. Although there were no statistically significant treatment-related effects on hatching success, growth or survival at any concentrations tested, there was a clear dose response at the highest concentration levels of 4.2 and 10 mg a.s./L in survival to day 28 post-hatch and less pronounced dose response in mean dry weight. Therefore, the NOEC of 1.7 mg a.s./L was set by RMS, based on survival to day 28 post-hatch. The LOEC was set 4.2 mg a. s. /L.

2.9.2.3.2 Chronic toxicity to aquatic invertebrates

One study performed with active substance daminozide was available (Last 2011). The study was not considered suitable for regulatory use since no NOEC could be determined and daminozide was tested simultaneously with formaldehyde therefore the results of the study reflect the combined toxicity of both substances, not only daminozide.

2.9.2.3.3 Chronic toxicity to algae or aquatic plants

Two valid studies performed with active substance daminozide were available, see point 2.9.2.2.3 above.

2.9.2.3.4 Chronic toxicity to other aquatic organisms

One study on *Lemna* carried out with active substance daminozide was available, see point 2.9.2.2.4 above.

2.9.2.4 Comparison with the CLP criteria

2.9.2.4.1 Acute aquatic hazard

Table 63: Summary of information on acute aquatic toxicity relevant for classification of active substance

Method	Species	Test material	Results[1]	Remarks	Reference
US EPA 72-2 (1975)	*Daphnia magna*	Daminozide	96 h EC_{50} 75.5 mg/L (mm)	-	Lintott (1992); A.7.4.1.8
OECD 201 (1984)	Freshwater green (*Pseudokirchneriella subcapitata*)	Daminozide	72 h E_rC_{50} >100 mg/L 72 h E_bC_{50} >100 mg/L (nom)	-	Manson & Scholey (2006); 2242/049-D2149
OECD 201 (2011)	Freshwater cyanobacteria (*Anabaena flos-aquae*)	Daminozide	72 h E_rC_{50} >100 mg/L 72 h EyC_{50} >100 mg/L (nom)	-	Seeland-Fremer & Mosch (2014); 87711210

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

2.9.2.4.2 Long-term aquatic hazard (including bioaccumulation potential and degradation)

Table 64: Summary of information on long-term aquatic toxicity relevant for classification of active substance

Method	Species	Test material	Results	Remarks	Reference
OECD 210 (1992), U.S. EPA OPPTS 850.1400	Fathead minnow (*Pimephales promelas*)	Daminozide	21 d NOEC 1.7 mg/L (mm)	-	Anonymous (2014);
OECD 201 (1984)	Freshwater green (*Pseudokirchneriella subcapitata*)	Daminozide	72 h NOEC 100 mg/L (nom)	-	Manson & Scholey (2006); 2242/049-D2149
OECD 201 (2011)	Freshwater cyanobacteria (*Anabaena flos-aquae*)	Daminozide	72 h NOEC 100 mg/L (nom)	-	Seeland-Fremer & Mosch (2014); 87711210

Chronic fish and algal toxicity data were available for active substance; the lower endpoint was derived from chronic fish ELS study (*Pimephales promelas*, NOEC = 1.7 mg a.s./L).

However, no valid chronic crustacean toxicity data neither for technical nor for formulated daminozide were available. Therefore, the following sentence from the ECHA Guidance on the application of CLP criteria (2009) can be applied in such case: "*It is this acute toxicity which has therefore been used as the core property in defining both the acute and the long-term hazard if no adequate chronic test data are available*." (see also Figure 4.1.1 of the Guidance). Thus, acute toxicity data were used as a surrogate system for defining long-term hazard for crustaceans (*Daphnia magna*, EC50 = 75.5 mg a.s./L).

Considering all the toxicity data available and the fact that daminozide is rapidly degradable substance and has log Kow <4, no aquatic chronic classification is required for daminozide.

2.9.2.5 Conclusion on classification and labelling for environmental hazards

No classification required for daminozide.

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

The Dossier Submitter (DS) proposed not to classify the substance as hazardous to the aquatic environment.

Degradation

Daminozide was hydrolytically stable in aqueous buffer solutions at pH 5 – 9.

Daminozide is stable to aqueous photolysis in sterilised irradiated pure water but can be photolytically degraded in irradiated sterilised natural waters. However, the rate of degradation (DT_{50}=36.8 days) was very slow compared to the degradation observed in water/sediment systems. The stability of daminozide to photolysis in pure waters, and the slow photolytic degradation in natural waters, is supported by the results of the UV/vis spectra, which indicate that daminozide does not absorb light with wavelengths >290 nm to a significant degree.

There is one ready biodegradability test available for daminozide following OECD TG 301E (Modified OECD Screening Test) using domestic activated sludge (adaptation not specified) resulting in 82% degradation after 28 days.

In an aerobic mineralisation in surface water study (OECD TG 309, GLP) 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. Daminozide degraded via an unidentified polar metabolite (maximum mean formation of 35.4 - 75.7% AR), to the terminal degradation product CO_2 (maximum formation 55.9 – 57.3% AR at study termination).

An aerobic water/sediment degradation study performed at 20°C in the dark demonstrated rapid biotic degradation of daminozide. The test was carried out in accordance with OECD TG 308 (draft) and in compliance with GLP. Whole system DT_{50} values of 0.88 days and 0.94 days were calculated in accordance with FOCUS Kinetics guidance. The only metabolite observed at concentrations > 5% AR in either in 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. Evolved $^{14}CO_2$ increased up to a maximum of 38.9% AR 7 days after application, whereupon a plateau was reached. Re-examination of the study demonstrates that the study only utilised HPLC analysis with radio- and UV detection. No confirmatory analytical method was reported and the only reference standard for potential polar metabolites investigated was that for formaldehyde. Therefore, though the formaldehyde standard eluted with a comparable retention time to the metabolite peak in the 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. The identification of the polar metabolite as formaldehyde within the study is therefore unreliable. Later it was confirmed that the unknown metabolite could only be methanol. The study demonstrated that in natural water/sediment systems, daminozide is likely to rapidly degrade to a polar metabolite (considered most likely to be methanol) which in turn is degraded to CO_2. For the polar metabolite, whole system DT_{50} values of 37.8 days and 93.4 days, with formation fractions of 0.283 and 0.258 were calculated.

Overall, the dominant route of degradation in natural aquatic systems is likely to be biotic, with daminozide degrading rapidly to methanol and then to CO_2. Photolytic degradation is unlikely to be significant.

In conclusion, the DS considered daminozide to be rapidly degradable, for classification purposes.

Bioaccumulation

The measured octanol-water partition coefficient (log K_{OW}) is – 1.53 at 20°C and pH 7. No bioaccumulation study is available for daminozide. The DS concluded that daminozide has a low potential to bioaccumulate in aquatic organisms.

Aquatic Toxicity

Reliable aquatic toxicity data for the active substance and a formulation are available in the CLP report, and a summary of the relevant information on aquatic toxicity is provided in the following Table (the key endpoints used in hazard classification are highlighted in bold). Additional data provided by the PPP Applicant during the renewal of the approval of the active substance are also presented in the Table. Results are expressed in terms of nominal concentrations, unless stated otherwise.

***Table**: Summary of relevant information on aquatic toxicity of daminozide*

Method	Test material	Species	Endpoint	Toxicity value (mg/L)	Reference
Short term toxicity					
OECD TG 203	Dazide Enhance SG (84.9% w/w daminozide)	*Cyprinus carpio*	96h LC_{50} (mortality)	420 (f) 357 (a.s.)	Anonymous (2009)
OECD TG 203	Dazide Enhance (85.5% w/w daminozide)	*Cyprinus carpio*	96h LC_{50} (mortality)	75.5 (f) **64 (a.s.)**	Anonymous (2010)
OECD TG 203	Alar 85 SG (86.1% w/w daminozide)	*Oncorhynchus mykiss*	96h EC_{50} (mortality)	36.1 (f) 31.1 (a.s.)	Anonymous (2017a)
OECD TG 202	Daminozide	*Daphnia magna*	96h EC_{50} 48h EC_{50} (immobility)	75.5 mm > 101 #	Lintott (1992)
OECD TG 202	Alar 85 SG (86.1% w/w daminozide)	*Daphnia magna*	48h EC_{50} (immobility)	76.5 (f) 65.9 (a.s.)	Kosak and Sonntag (2017)
OECD TG 201	Daminozide	*Pseudokirchneriella subcapitata*	72h E_rC_{50} 72h E_bC_{50} (growth inhibition)	>100 >100	Manson and Scholey (2006)
OECD TG 201	Alar 85 SG (86.1% w/w daminozide)	*Pseudokirchneriella subcapitata*	72h E_rC_{50} 72h E_yC_{50} (growth inhibition)	65.4 (f) 56.3 (a.s.) 28.3	Hermes and Emnet (2017b)
OECD TG 201	Daminozide	*Anabaena flos-aquae*	72h E_rC_{50} 72h E_yC_{50} (growth inhibition)	>100 >100	Seeland-Fremer and Mosch (2014)

OECD TG 221	Alar 85 SG (86.1% w/w daminozide)	*Lemna gibba*	7d E_rC_{50} 7d E_yC_{50} (growth inhibition)	171 (f) 147.2 (a.s) 88.2	Hermes and Emnet (2017c)
OECD TG 239	Alar 85 SG (86.1% w/w daminozide)	*Myriophyllum spicatum*	14d E_rC_{50} 14d E_yC_{50} (total shoot length (cm)) 14d E_rC_{50} 14d E_yC_{50} (shoot fresh weight (g)) 14d E_rC_{50} 14d E_yC_{50} (shoot dry weight (g))	> 100 > 100 > 100 > 100 > 100 > 100	Schwarz (2020)
Long term toxicity					
OECD TG 210, U.S. EPA OPPTS 850.1400	Daminozide	*Pimephales promelas*	21d NOEC (growth, mortality)	**1.7 mm**	Anonymous (2014)
OECD TG 211	Daminozide	*Daphnia magna*	21d NOEC 21d EC_{10} (mortality, reproduction)	≥100 >100	Börschig and Wydra (2018)
OECD TG 201	Daminozide	*Pseudokirchneriella subcapitata*	72h NOEC (growth inhibition)	100	Manson and Scholey (2006)
OECD TG 201	Alar 85 SG (86.1% w/w daminozide)	*Pseudokirchneriella subcapitata*	72h NOE_rC 72h NOE_yC (growth inhibition)	6.3 2.0	Hermes and Emnet (2017b)
OECD TG 201	Daminozide	*Anabaena flos-aquae*	72h NOEC (growth inhibition)	100	Seeland-Fremer and Mosch (2014)
OECD TG 221	**Alar 85 SG (86.1% w/w**	***Lemna gibba***			Hermes and Emnet

	daminozide)		7d NOE_rC 7d NOE_yC (growth inhibition)	20.0 20.0	(2017c)
OECD TG 239	Alar 85 SG (86.1% w/w daminozide)	*Myriophyllum spicatum*	14d NOE_rC 14d NOE_yC (total shoot length (cm)) 14d EC_{10} 14d NOE_rC 14d NOE_yC (shoot fresh weight (g)) 14d EC_{10} 14d NOE_rC 14d NOE_yC (shoot dry weight (g))	100 31.3 68 100 100 93.1 100 > 100 100	Schwarz (2020)

Note: mm – mean measured concentrations; (f) – formulation; (a.s.) – active substance; # provided by PPP Applicant during the procedure for renewal of the approval of daminozide; Th results were not included in the CLH report, but only in latter stages of the CLH case processing

Acute toxicity

In addition to studies using the active substance daminozide, studies using daminozide formulations are presented in the CLH report.

For acute aquatic toxicity, reliable toxicity data for the active substance are reported for invertebrates and algae, while reliable data for fish are lacking. Due to the lack of acute toxicity data for active substance for the fish, the DS used the LC_{50} of 64 mg/L derived from semi-static acute toxicity study performed according to OECD TG 203 with the formulation Dazide Enhance (85.5% w/w daminozide) on common carp (*Cyprinus carpio*) as the basis for classification, as this is the most sensitive acute endpoint. In this study, all analytical measurements were between 90-104% of nominal concentrations. Based on this lowest effect value, the DS proposed not to classify daminozide as acutely hazardous to the aquatic environment.

Chronic toxicity

In the CLH report, only studies performed with the active substance are available. Reliable long-term aquatic toxicity data are available for fish and algae, while data for crustacean are lacking. From the available aquatic toxicity data, fish are the most

sensitive trophic group. The lowest chronic endpoint was a 21d mean measured NOEC of 1.7 mg/L, reported for *Pimephales promelas*. Due to the lack of chronic toxicity data for the crustacean, the DS used the surrogate approach (96h EC_{50} of 75.5 mg/L for *D. magna*). Considering all toxicity data available and the fact that daminozide is rapidly degradable and has low potential for bioaccumulation, this results in no classification for chronic aquatic hazard.

Comments received during public consultation

Comments were received from four MSCAs and one company-manufacturer. Three MSCAs and one company-manufacturer agreed with DS proposal not to classify the substance as hazardous to the aquatic environment. One MSCA questioned the relevance of the chronic fish toxicity study performed with the formulation 'Dazide Enhance' for the classification of the active substance due to presence of co-formulants. The DS explained that co-formulants present in formulation in amount >0.01% w/w do not require any classification. Therefore, their influence on the toxicity of daminozide is not considered relevant. RAC notes that the comment refers to the acute fish toxicity study.

Another MSCA pointed out that due to fact that daminozide is a plant growth regulator, it would be useful to have toxicity data on aquatic macrophytes in order to confirm the environmental classification of daminozide. The DS responded that the study has already been performed by the PPP applicant and it will be submitted and used for classification during the on-going peer-review process.

Assessment and comparison with the classification criteria

Degradation

RAC agrees with the DS proposal to consider daminozide as rapidly degradable. The substance is readily biodegradable (82% degradation after 28 days), subject to rapid biotic degradation in an aerobic mineralisation study (DT_{50}=0.13 – 0.15 days) and a water/sediment simulation study (DT_{50}=0.88 - 0.94 days for whole system). The degradation product (considered most likely to be methanol) was formed in aerobic mineralisation and water/sediment studies and there was a clear evidence of rapid degradation to CO_2. Therefore, the substance is considered to be rapidly degradable for the purposes of environmental classification.

Bioaccumulation

RAC agrees with DS that daminozide has a low potential to bioaccumulate in aquatic organisms. The basis for this is the experimentally derived Log K_{OW} value of – 1.53, which is below the CLP Regulation threshold of 4.

Aquatic Toxicity

Acute toxicity

In the CLP report, reliable short-term aquatic toxicity data with the active substance are available for two trophic levels, invertebrates and algae, while data for fish are lacking. During the opinion development process, additional studies performed with formulation

Alar 85 SG for all three trophic levels (fish, daphnia, algae and aquatic plants) were provided by the PPP Applicant (see Table). RAC evaluated these additional studies and considers them of adequate scientific quality.

In the aquatic acute toxicity studies performed with the formulation, slightly higher toxicity to the invertebrate *Daphnia magna* (48h EC_{50}=65.9 mg daminozide/L) and algae *Pseudokirchneriella subcapitata* (72h E_rC_{50}=56.3 mg daminozide/L) was observed, compared to studies performed for the same species only with the active substance. In all cases, the toxicity exceeds considerably the regulatory threshold value of 1 mg/L.

RAC notes that no information has been provided about any co-formulants and their potential impact on the study outcome. Thus, due to the uncertainties related to the data on the formulation, RAC decided to consider these studies on the formulated product only as additional supporting information and base the decision on (no) aquatic acute hazard classification on data on the active substance.

Overall, RAC is of the opinion that adequate acute toxicity data with the active substance are available for two trophic levels (daphnia and algae). Since all $L(E)C_{50}$ values for invertebrates and algae (see Table) are above the threshold value of 1 mg/L, daminozide does not meet the criteria for classification for acute aquatic hazard. Consequently, RAC agrees with the DS that **daminozide does not warrant classification for acute aquatic hazard.**

A review of the classification might be necessary if data for the missing trophic level become available.

Chronic toxicity

In the CLP report, reliable long-term aquatic toxicity data with the active substance are reported for two trophic levels, fish and algae.

During the opinion development process, a new *Daphnia magna* reproduction test (Börschig and Wydra, 2018) performed with the active substance was provided by the PPP Applicant. RAC assessed the new study and considers it as valid and reliable and that it should be taken into account for classification purposes.

Additionally, during the opinion development process, new studies performed with the formulation Alar 85 SG for aquatic plants (*Lemna gibba* and *Myriophyllum spicatum*) were provided by the PPP Applicant. The outcome of these studies (see Table) also show toxicity values above the regulatory threshold value for classification of the substance as chronic hazardous to the aquatic environment.

In line with the argumentation on the use of the acute aquatic studies for formulation Alar 85 SG, RAC decided to also consider the chronic studies on the formulated product only as additional supporting information, due to uncertainties introduced by co-formulants and their potential impact on the study outcome.

Overall, RAC is of the opinion that adequate chronic toxicity data with the active substance are available for all three trophic levels (fish, daphnia and algae). Fish are the most sensitive group and the lowest result is a 21d NOEC value of 1.7 mg/L for fathead minnow, *Pimephales promelas*. As discussed previously, daminozide is rapidly degradable and has low potential to bioaccumulation. Based on this, daminozide does not fulfil the criteria for chronic hazard classification. Therefore, RAC agrees with DS that **daminozide does not warrant classification for chronic aquatic hazard.**

Supplemental information - In depth analyses by RAC

During the process of the preparation of the first draft opinion, RAC became aware of additional information generated during the procedure for renewal of the approval of daminozide (January 2020). This additional information was submitted in February 2020 and assessed by RAC during the preparation of this opinion. The additional information provided by the PPP Applicant included data re-evaluations, clarifications on endpoints and seven new experimental studies performed with active substance (one study) and formulation Alar 85 SG (six studies).

Acute Toxicity to Rainbow Trout (Oncorhynchus mykiss) in a 96-hour Static Test (Anonymous, 2017a)

Rainbow trout (*Oncorhynchus mykiss*) were exposed to the formulation Alar 85 SG (86.1% w/w daminozide) for 96 hours under static exposure conditions to the nominal concentrations of 6.4, 14.1, 31.0, 68.2 and 150 mg/L. During the test, the fish were exposed to a mean test item concentration of 111% of the nominal values. The test was carried out in accordance with OECD TG 203 and in compliance with GLP. The validity criteria were met. The 96 hour nominal LC_{50} (mortality) was 36.1 mg formulation/L (equivalent to 31.1 mg daminozide/L).

Acute Toxicity to Daphnia magna in a Static 48-hour Immobilisation Test (Kosak and Sonntag, 2017)

The freshwater crustacean *Daphnia magna* was exposed to the formulation Alar 85 SG (86.1% w/w daminozide) for 48 hours under static exposure conditions to the nominal concentrations of 4.3, 9.4, 20.7, 45.4 and 100 mg/L. During the test, the daphnids were exposed to a mean test item concentration of 95% of the nominal values. The test was carried out in accordance with OECD TG 202 and in compliance with GLP. The validity criteria were met. In the study, the pH value of the test media used for the different experimental treatments varied by more than 1.5 units at the start of the test, but this does not invalidate the study since all validity criteria were met. The 48 hour nominal EC_{50} was 76.5 mg formulation/L (equivalent to 65.9 mg daminozide/L).

Toxicity to Pseudokirchneriella subcapitata in an Algal Growth Inhibition Test (Hermes and Emnet, 2017b)

The freshwater green algae *Pseudokirchneriella subcapitata* was exposed to the formulation Alar 85 SG (86.1% w/w daminozide) for 72 hours under static exposure conditions to the nominal concentrations of 0.2, 0.6, 2.0, 6.3, 20.0, 63.3 and 200 mg/L. During the test, the algae were exposed to a mean test item concentration of 101% of the nominal values. The test was carried out in accordance with OECD TG 201 and in compliance with GLP. The pH in the control treatment increased by more than 1.5 units over the study period although this is not considered to have impacted the test results. The validity criteria were met. Based on nominal concentrations, the 72 hour E_rC_{50} was 65.4 mg formulation/L (equivalent to 56.3 mg daminozide/L), 72 hour NOE_rC was 6.3 mg formulation/L, 72 hour E_yC_{50} was 28.3 mg formulation/L and 72 hour NOEyC was 2.0 mg formulation/L.

Toxicity to the Aquatic Plant Lemna gibba in a Static Growth Inhibition Test (Hermes and Emmet, 2017c)

The freshwater plant *Lemna gibba* was exposed to the formulation Alar 85 SG (86.1% w/w daminozide in the study/85% in document MCP 10.2.1.1.) for 7 days under static exposure conditions to the nominal concentrations of 0.2, 6.3, 20.0, 63.3 and 200 mg/L. During the test the *Lemna* were exposed to a mean test item concentration of 89 % of the nominal values. Test was carried out in accordance with OECD TG 221 and GLP. The validity criteria were met. Based on nominal concentrations, the 7 day E_rC_{50} was 171 mg formulation/L (equivalent to 147.2 mg daminozide/L), 7 day NOE_rC was 20.0 mg/L, 7 day E_yC_{50} was 88.2 mg/L and 7 day NOE_yC was 20.0 mg/L.

Growth Inhibition of Myriophyllum spicatum in a Water/Sediment System (Schwarz, 2020)

The 14 day, GLP study followed OECD TG 239: Water-Sediment *Myriophyllum spicatum* Toxicity Test (26-Sep-2014). Rooted aquatic macrophyte, *Myriophyllum spicatum* was exposed to five nominal concentrations of the formulation Alar 85 SG (86.1% w/w daminozide) of 0.954, 3.05, 9.77, 31.3 and 100 mg/L for 14 days in a static water-sediment system under controlled environment conditions. The test item was spiked to the water. The validity criteria were met. For all test item concentrations, the geometric mean concentrations were within 20% of the nominal concentrations. For all toxicological endpoints, no effects > 40% were determined. Due to these facts, the evaluation was conducted using nominal concentrations of the test item. A summary of the results on aquatic toxicity of this formulation to *M. spicatum* is provided in the following Table. The results are expressed in terms of nominal concentrations.

***Table**: Summary of effects following a 14 day exposure of the aquatic macrophyte Myriophyllum spicatum*

Endpoint	Toxicity value (mg/L)
total shoot length (cm)	14-day E_rC_{50}>100 14-day NOE_rC=100 14-day E_yC_{50}>100 14-day NOE_yC=31.3
shoot fresh weight (g)	14-day E_rC_{50}>100 14-day EC_{10}=68 14-day NOE_rC=100 14-day E_yC_{50}>100 14-day NOE_yC=100

shoot dry weight (g)	14-day E_rC_{50}>100 14-day EC_{10}=93.1 14-day NOE_rC=100 14-day E_yC_{50}>100 14-day NOE_yC=100

Effect on Daphnia magna in a Semi-Static Reproduction Test (Börschig and Wydra, 2018)

The crustacean *Daphnia magna* was exposed to active substance daminozide for 21 days under semi-static exposure conditions to the nominal concentrations of 100, 32, 10, 3.2 and 1.0 mg/L. Concentrations of the test item were within ±20 % of the nominal concentrations during the test. Test was carried out in accordance with OECD TG 211 and in compliance with GLP. The validity criteria were met. The 21 day nominal NOEC of ≥100 mg/L and 21 day nominal EC_{10} of ≥100 mg/L for effect on mortality and reproduction were determined.

Additional information for acute toxicity study (Lintott, 1992)

The 48 hours EC_{50} value of > 101 mg daminozide/L was provided by the PPP Applicant for acute toxicity study for *D. magna* (Lintott, 1992). In the view of RAC, the 48 hour EC_{50}, rather than 96 hour EC_{50}, should be selected as the lowest value for this species and for classification purposes. This is also in line with CLP guidance (Version 5.0 – July 2017, section I.2.2.1): "For daphnids, test duration of 48 hours is used."

Overall, RAC notes that new information provided by the PPP Applicant supports no classification of daminozide as hazardous to the aquatic environment as proposed by DS.

2.9.3 Summary of effects on arthropods

Effects on bees

Table 65: Summary of reported laboratory bee toxicity studies with technical and formulated daminozide

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

Study evaluated in old DAR (1999).

Effects on other arthropods

Table 66: Laboratory tests with non-target arthropods

Species	Life stage	Test substance	Study type	Dose (kg /ha)[2]	Mortality/ Corr. mortality (%)	Sublethal effects[3]	References
Laboratory tests							
Aphidius rhopalosiphi	Adult	Alar 85 SP	Tier I Glass plate Limit test	Control 10 form. (8.5 a.s.)	2.5 12.5 / 10 LR_{50} >10 kg form./ha (>8.5 kg a.s./ha)	No. of pupae / % adverse effects 21.1 22.6 / -7.1% ER_{50} >10 kg form./ha (>8.5 kg a.s./ha)	Baxter 1999a; UNI-99-9
Typhlodromus pyri	Protonymph[1]	Daminozide	Tier I Glass plate Limit test	Control 7.225 a.s.	n.a.	n.a.	Harwood 2000; 18099

Species	**Life stage**	**Test substance**	**Study type**	**Dose (kg /ha)[2]**	**Mortality/ Corr. mortality (%)**	**Sublethal effects[3]**	**References**
	Protonymph[1]	Dazide 85	Tier I Glass plate Limit test	Control 10 form. (8.5 a.s.)	n.a.	n.a.	Harwood 2000; 18133
	Protonymph	Alar 85 SP	Tier I Glass plate Limit test	Control 10 form. (8.5 a.s.)	3 14 / 11.3 LR_{50} >10 kg form./ha (>8.5 kg a.s./ha)	No. of eggs per female / % adverse effects 7.2 3.9 / 45.8% ER_{50} >10 kg form./ha (>8.5 kg a.s./ha)	Vinall 1999; UNI-99-8
Encarsia formosa	Adult	Alar 85 SP	Tier I Glass plate Limit test	Control 10 form. (8.5 a.s.)	18 85 / 82 LR_{50} <10 kg form./ha (<8.5 kg a.s./ha)	No. of parasitized scales / % adverse effects 18.2 17.8/ 2.2% ER_{50} >10 kg form./ha (>8.5 kg a.s./ha)	Halsall 2000; UNI-00-2
Orius laevigatus	Adult	Alar 85 SP	Tier I Glass plate Limit test	Control 10 form. (8.5 a.s.)	17 14 / 0 LR_{50} >10 kg form./ha (>8.5 kg a.s./ha)	No. of eggs per female / % adverse effects 7.5 7.9 / -5.3% ER_{50} >10 kg form./ha (>8.5 kg a.s./ha)	Vinall 2000; UNI-00-3
Poecilus cupreus	Adult	Alar 85 SP	Tier I Glass plate Limit test	Control 10 form. (8.5 a.s.)	0 0 / 0 LR_{50} >10 kg form./ha (>8.5 kg a.s./ha)	No. of larvae consumed / % adverse effects 4.83 4.90 / -1.4% ER_{50} >10 kg form./ha (>8.5 kg a.s./ha)	Baxter 1999b; UNI-99-10

Species	**Life stage**	**Test substance**	**Study type**	**Dose (kg /ha)[2]**	**Mortality/ Corr. mortality (%)**	**Sublethal effects[3]**	**References**
Chrysoperla carnea	Larva	Alar 85 SP	Tier I Glass plate Limit test	Control 10 form. (8.5 a.s.)	10 12 / 2 LR_{50} >10 kg form./ha (>8.5 kg a.s./ha)	No. of eggs per female / % adverse effects 15.7 15.4 / 1.9 ER_{50} >10 kg form./ha (>8.5 kg a.s./ha)	Barton 1999; UNI-99-11
Extended laboratory tests							
Typhlodromus pyri	Protonymph	Alar 85 SP	Tier I Glass plate	Control 5 form. (4.25 a.s.) 10 form. (8.5 a.s.)	19 23 / 5 15 / 0 LR_{50} >10 kg form./ha (>8.5 kg a.s./ha)	No. of eggs per female / % adverse effects 5.3 6.0 / -13.2% 5.1 / 3.8% ER_{50} >10 kg form./ha (>8.5 kg a.s./ha)	Taruza 2001a; UNI-01-1
Typhlodromus pyri	Protonymph	Dazide 85	Tier I Glass plate	Control 1.176 form. (1.0 a.s.) 4.412 form. (3.75 a.s.) 8.824 form. (7.5 a.s.)	14 18 / 5 21 / 8 36 / 26 LR_{50} >8.824 kg form./ha (>7.5 kg a.s./ha)	No. of eggs per female / % adverse effects 8.1 8.1 / 0% 7.6 / 6.2% 6.5 / 19.8% ER_{50} >8.824 kg form./ha (>7.5 kg a.s./ha)	Taruza 2001b; RIV-02-1

[1] *the study is not considered valid*
[2] *form. – formulation; a.s. - active substance*
[3] *positive percentages relate to adverse effects in comparison with control*
n.a. – not applicable

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

2.9.4 Summary of effects on non-target soil meso- and macrofauna

Earthworms

Table 67: Summary of studies on toxicity to earthworms

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

** The highest concentration tested.*

2.9.5 Summary of effects on soil nitrogen transformation

Table 68: Summary of data on the toxicity of daminozide to soil micro-organisms

Test	Test substance	Endpoint	Reference
Nitrogen [# 1], mineralisation	Alar 85	n.a.	Mass (1987 & 1989) A.8.1.18

[#] *Study evaluated in old DAR (1999).*
[1] *The study is not considered valid or suitable for regulatory use.*

No valid endpoint for soil nitrogen transformation was available.

2.9.6 Summary of effects on terrestrial non-target higher plants

Table 69: Effects of daminozide on non-target plants

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

** The highest concentration tested.*

2.9.7 Summary of effects on other terrestrial organisms (flora and fauna)

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2.9.8 Summary of effects on biological methods for sewage treatment

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2.9.9 Summary of product exposure and risk assessment

A risk assessment for non-target organisms is presented for daminozide in the Dazide Enhance formulation (code FAL 2400; synonymous with Dazide 85 WG, Dazide WG, Dazide SG), and in the Alar formulation (synonymous with B-NINE, Alar 85 SG, Daminozide SG). Both representative formulations are water soluble granule formulations (SG) containing 850 g/kg daminozide. The products are a plant growth regulators intended for use on field and protected ornamental plants. The mode of action is through interference with gibberellic acid biosynthesis. It is absorbed by the leaves and translocated throughout the treated plant. As a result more compact plants (by inhibition of intermodal elongation) are produced.

Intended application pattern

The use pattern for both representative formulations is summarised below.

Table 70: Intended application pattern

Crop	Timing of application BBCH	Method of application	Number of applications	Interval between applications (min.)	Maximum application rate, individual treatment	
					Product [kg/ha]	Daminozide [kg a.s./ha]
Ornamentals (Protected)	<50	Over spray (Gantry)	1 - 5	7 days	9.0	7.65
Ornamentals (Field)	<50	Foliar*	1 - 5	7 days	5.0	4.25

* Application using a knapsack sprayer

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

Representative formulations used for Annex I inclusion were Dazide 85 (SP) and Alar 85 (UBI 2231-01 SP), the earlier formulations of the current ones. The differences in composition among all these formulations are considered as minor and their toxicities are considered to be comparable. For detailed composition of all these formulations see Volume 4 Annex C.

2.9.9.1 Risk assessment for birds and other terrestrial vertebrates

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

The risk assessment was performed for field use (4.25 kg a.s./ha). For protected use (7.65 kg a.s./ha), the risk assessment for birds and mammals should be performed assuming the same exposure as for a field use, unless it is

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

2.9.9.1.1 Risk assessment for birds

Screening assessment

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

Table 71: Avian screening assessment for the proposed use of daminozide on ornamentals

Crop	Indicator spp.	Time scale & shortcut value	MAF	TWA	DDD (mg/kg bw)	Endpoint (mg/kg bw)	TER	Trigger value
Field use (application rate: 5 x 4.25 kg a.s./ha with 7 day interval)								
Ornamentals	Small insectivorous bird	Acute: 46.8	1.9	-	378	4248 [a]	11.2	10
		Long-term: 18.2	2.4	0.53	98.4	79.9	**0.81**	5
Protected use (application rate: 5 x 7.65 kg a.s./ha with 7 day interval)								
Ornamentals	Small insectivorous bird	Acute: 46.8	1.9	-	680	4248 [a]	**6.2**	10
		Long-term: 18.2	2.4	0.53	177	79.9	**0.45**	5

[a] Extrapolated from the reported endpoint of >2250 mg a.s./kg bw, based on a factor of 1.888
MAF: Multiple application factor; TWA: time weighted average; DDD: daily dietary dose
Value(s) in bold are below the trigger value

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

Tier I assessment

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

Table 72: Tier I TER values for birds foraging in treated ornamentals

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

MAF: Multiple application factor; TWA: time weighted average; DDD: daily dietary dose
Value(s) in bold are below the trigger value

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

Refined long-term dietary risk assessment

The Notifier provided the refined long-term dietary risk assessment (see Volume 3 CP B.9).

RMS agrees to use the blue tit (*Cyanistes caeruleus*) as a specific focal specis. It is identified in EFSA GD (2009) as the representative species for ornamentals with canopy directed application and it is a widespread and common species throughout the Europe. Its primary habitat is deciduous woodland but it also occurs in coppice, overgrown marshes and mires etc. The species is frequent in parks, gardens and other man-made habitats (Aagaard, 2014). In addition, the blue tit is considered sufficiently protective also for other species due to its low body weight.

RMS agrees to use PD of 1.

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

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

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

Refined long-term risk assessment: TER calculation

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

Table 73: Refined TER value for small insectivorous birds (blue tit) foraging in treated ornamentals

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

[a] FIR/bw: food intake rate per body weight according to EFSA (2009)
[b] RUD: residues per unit dose according to EFSA (2009)
DF: deposition factor
MAF: multiple application factor
f_{TWA}: time weighted average factor
PT: proportion of diet obtained in the treated area
PD: proportion of food type in the diet
AR: single application rate
DDD: daily dietary dose

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

Dietary risk to birds from metabolites

No studies on residues in plants were available.

As methanol was identified as a potentially relevant metabolite in surface water and soil compartments, potential exposure of birds to this metabolite should be assessed. No toxicity datawere available for the metabolite methanol. However, based on the physical-chemical properties of methanol; i.e. high vapour pressure (1.69×10^4 Pa at 25ºC) from soil, it is assumed that methanol will be present in relevant food items in rather small amounts. Therefore, the exposure of birds to methanol is expected to be negligible and the risk is considered to be covered with the risk assessment for the parent daminozide. No special dietary risk assessment for methanol is required.

Risk assessment for drinking water exposures

Puddle scenario

According to the EFSA guidance document an assessment for puddle scenario is not required when the ratio of effective application rate (in g/ha) to the relevant endpoint (in mg/kg bw/d) does not exceed 50 in the case of less sorptive

substances (Koc < 500 L/kg) or 3000 where the Koc ≥500 L/kg.

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

Table 74: Ratios of effective application rate to endpoints for daminozide and its metabolite

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

[a] *Based on the geomean soil DT_{50} of 0.12 days*
[b] *Extrapolated from the reported endpoint of >2250 mg a.s./kg bw, based on a factor of 1.888*
[c] *Based on the soil DT_{50} of 3.9 days (geomean)*
[d] *There are no toxicity data available for the metabolites methanol, therefore the ametabolite has been assumed to be 10 times more toxic than the parent (LD50 = 4248 mg a.s./kg bw / 10 = 424.8 mg a.s./kg bw).*
[e] *There are no toxicity data available for the metabolite methanol, therefore the ametabolite has been assumed to be 10 times more toxic than the parent (NOEL = 79.7 mg a.s./kg bw/d / 10 = 7.97 mg a.s./kg bw/d).*
MAF: Multiple application factor

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

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

Table 75: Tier I avian drinking water assessment (puddle scenario) for the proposed use of daminozide

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

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

Risk for Bioaccumulation and Secondary Poisoning

As the log Pow of daminozide and methanol are less than the trigger value of 3 (log Pow at pH 7 = -1.5 and -0.77[1], respectively), the risk to birds from secondary poisoning is consider to be negligible and no further consideration is required.

Conclusion – risk to birds

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

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

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

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

High risk from drinking water exposure was identified for methanol.

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

2.9.9.1.2 Risk assessment for other terrestrial vertebrates

Screening assessment

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

Table 76: Mammal screening assessment for the proposed use of daminozide on ornamentals

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

[a] *Extrapolated from the reported endpoint of >2250 mg a.s./kg bw, based on a factor of 1.888*
MAF: Multiple application factor; TWA: time weighted average; DDD: daily dietary dose
Value(s) in bold are below the trigger value

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

Table 77: Tier I TER values for mammals foraging in treated ornamentals

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

Small omnivorous mammal "mouse"	Application crop directed BBCH 10-49	Acute: 17.2	1.9	-	250	>5000	>20.00	10
		Long-term: 7.8	2.4	0.53	75.90	500	6.59	5

MAF: Multiple application factor; TWA: time weighted average; DDD: daily dietary dose
Value(s) in bold are below the trigger value

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

Refined long-term dietary risk assessment

The Notifier proposed the refined long-term dietary risk assessment (see Volume 3 CP B.9).

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

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

RMS agrees to use PT of 1.

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

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

Table 78: Refined acute TER values for small herbivorous mammal (common vole) foraging in treated ornamentals

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

MAF: multiple application factor
PT: proportion of diet obtained in the treated area
PD: proportion of food type in the diet
AR: single application rate
DDD: daily dietary dose
Value(s) in bold are below the trigger value

Table 79: Refined long-term TER values for small herbivorous mammal (common vole) foraging in treated ornamentals

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

[a] *FIR/bw: food intake rate per body weight according to EFSA (2009)*
[b] *RUD: residues per unit dose according to EFSA (2009)*
DF: deposition factor
MAF: multiple application factor
f_{TWA}: time weighted average factor
PT: proportion of diet obtained in the treated area
PD: proportion of food type in the diet
AR: single application rate
DDD: daily dietary dose
Value(s) in bold are below the trigger value

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

Dietary risk to mammals from metabolites

No studies on residues in plants were available.

As methanol was identified as a potentially relevant metabolite in surface water and soil compartments, potential exposure of mammals to this metabolite should be assessed. No toxicity datawere available for the metabolite methanol. However, based on the physical-chemical properties of methanol; i.e. high vapour pressure (1.69×10^4 Pa at 25ºC) from soil, it is assumed that methanol will be present in relevant food items in rather small amounts. Therefore, the exposure of mammals to methanol is expected to be negligible and the risk is considered to be covered with the risk assessment for the parent daminozide. No special dietary risk assessment for methanol is required.

Risk assessment for drinking water exposures

Puddle scenario

According to the EFSA guidance document an assessment for puddle scenario is not required when the ratio of effective application rate (in g/ha) to the relevant endpoint (in mg/kg bw/d) does not exceed 50 in the case of less sorptive

substances (Koc < 500 L/kg) or 3000 where the Koc ≥500 L/kg.

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

Table 80: Ratios of effective application rate to endpoints for daminozide and its metabolite

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

[a] *Based on the geomean soil DT_{50} of 0.12 days*
[c] *Based on the soil DT_{50} of 3.9 days (geomean)*
[d] *There are no toxicity data available for the metabolites methanol, therefore the ametabolite has been assumed to be 10 times more toxic than the parent (LD50 >5000 mg a.s./kg bw / 10 = >500 mg a.s./kg bw).*
[e] *There are no toxicity data available for the metabolite methanol, therefore the ametabolite has been assumed to be 10 times more toxic than the parent (NOEL = 500 mg a.s./kg bw/d / 10 = 50 mg a.s./kg bw/d).*
MAF: Multiple application factor

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

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

Table 81: Tier I drinking water assessment (puddle scenario) for the proposed use of daminozide

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

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

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

Risk for Bioaccumulation and Secondary Poisoning

As the log Pow of daminozide and methanol are less than the trigger value of 3 (log Pow at pH 7 = -1.5 and -0.77[2], respectively), the risk to mammals from secondary poisoning is consider to be negligible and no further consideration is required.

Conclusion – risk to vertebrates other than birds

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

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

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

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

No risks were identified for methanol.

2.9.9.2 Risk assessment for aquatic organisms

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

[2] *Material Safety Data Sheet – Methanol (CAS # 67-56-1). https://fscimage.fishersci.com/msds/14280.htm*

ANNEX 1 – BACKGROUND DOCUMENT TO RAC OPINION ON DAMINOZIDE (ISO); 4-(2,2-DIMETHYLHYDRAZINO)-4-OXOBUTANOIC ACID; N-DIMETHYLAMINOSUCCINAMIC ACID

Endpoints used in risk assessment

Table 82: Endpoints of technical and formulated daminozide and its metabolite used in risk assessment

Test organism	Test substance	Time scale, study type	Endpoint	Toxicity (mg a.s./L)	Reference
FISH					
Common carp (*Cyprinus carpio*)	Dazide Enhance SG	Acute, 96h (semi-static)	Mortality, LC_{50}	420 form. 357 a.s. (nom)	Anonymous (2009);
Common carp (*Cyprinus carpio*)	Dazide Enhance	Acute, 96h (semi-static)	Mortality, LC_{50}	75 form. 64 a.s. (nom)	Anonymous (2010
Fathead minnow (*Pimephales promelas*)	Daminozide	Chronic, 33d ELS (flow-through)	Development and growth, NOEC	1.7 (mm)	Anonymous (2014);
AQUATIC INVERTEBRATES					
Daphnia magna	Daminozide	Acute, 96h (flow-through)	Immobility, EC_{50}	75.5 (mm)	Lintott (1992); A.7.4.1.8
Daphnia magna	Dazide Enhance	Acute, 48h (static)	Immobility, EC_{50}	60 form. 51 a.s. (nom)	Goodband & Mullee (2010); 41004366
Daphnia magna	Dazide Enhance SG	Acute, 48h (static)	Immobility, EC_{50}	>100 form. >85 a.s. (nom)	Hernádi (2007); 07/482-023DA
ALGAE					
Freshwater green (*Pseudokirchneriella subcapitata*)	Daminozide	72 h (static)	Growth rate: E_rC_{50}	>100 (nom)	Manson & Scholey (2006); 2242/049-D2149
Freshwater cyanobacteria (*Anabaena flos-aquae*)	Daminozide	72 h (static)	Growth rate: E_rC_{50}	>100 (nom)	Seeland-Fremer & Mosch (2014); 87711210
Freshwater green (*Pseudokirchneriella subcapitata*)	Dazide Enhance SG	72 h (static)	Growth rate: E_rC_{50}	>100 form. >85 a.s. (nom)	Hernádi (2007); 07/482-022AL
AQUATIC PLANTS					
-					
Potential endocrine disrupting properties (Annex Part A, point 8.2.3) -					
(nom) nominal concentration; (mm) mean measured concentration; form.: formulation; a.s.: active substance n.a. not applicable					

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

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

Toxicity exposure ratios for aquatic species for active substance and its metabolites

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

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

Bold figures fall below the Regulation (EU) 546/2011trigger value
[a]PEC_{sw} for a single application as a worse case
**[Only scenarios where the trigger is not met at FOCUSsw step 1-2 should be included in step 3.]*

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

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

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

Bold figures fall below the Regulation (EU) 546/2011trigger value
[a] PEC_{sw} for a single application as a worse case
**[Only scenarios where the trigger is not met at FOCUSsw step 1-2 should be included in step 3.]*

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

Toxicity/exposure ratios for the most sensitive aquatic organisms (Regulation (EU) N° 284/2013, Annex Part A, point 10.2):

TERs for daminozide – ornamentals (glasshouse/indoor use) at 7.65 kg a.s./ha x 5

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

Bold figures fall below the Regulation (EU) 546/2011trigger value
[a]PEC_{sw} for a single application as a worse case
**[Only scenarios where the trigger is not met at FOCUSsw step 1-2 should be included in step 3.]*

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

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

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

Bold figures fall below the Regulation (EU) 546/2011trigger value
[1] There are no valid toxicity data available for the metabolite methanol, therefore it was assumed to be 10 times more toxic than the parent.
**[Only scenarios where the trigger is not met at FOCUSsw step 1-2 should be included in step 3.]*

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

Toxicity/exposure ratios for the most sensitive aquatic organisms (Regulation (EU) N° 284/2013, Annex Part A, point 10.2):

$FOCUS_{sw}$ step 1-2 - TERs for metabolite methanol – ornamentals >50 cm (field use) at 4.25 kg a.s./ha x 5

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

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

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

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

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

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

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

Bold figures fall below the Regulation (EU) 546/2011trigger value
[1] There are no valid toxicity data available for the metabolite methanol, therefore it wase assumed to be 10 times more toxic than the parent.
**[Only scenarios where the trigger is not met at FOCUSsw step 1-2 should be included in step 3.]*

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

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

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

Risk to aquatic life from metabolite contamination of groundwater

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

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

Conclusion – risk to aquatic organisms

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

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

2.9.10 Risk assessment for arthropods

2.9.10.1 Risk assessment for bees

EFSA Guidance on the risk assessment of plant protection products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees) (EFSA Journal 2013;11(7):3295) was published already in July 2013, but it has not come into force yet. However, based on the Technical report on the outcome of the pesticides peer review meeting on general recurring issues in ecotoxicology (EFSA, 2015), the risk assessment for bees (first tier) should be carried out according to EFSA Guidance, therefore it has been used in the present risk assessment.

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

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

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

Risk assessment for honeybees:

1) Field use

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

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

Figures in bold exceed the relevant trigger value

Risk assessment for honeybees from consumption of contaminated water

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

Figures in bold exceed the relevant trigger value

Both acute adult $HQ_{contact}$ and $ETR_{acute\ adult\ oral}$ values for formulation did not meet the relevant triggers at screening assessment. However, the acute oral and contact LD_{50} values were derived from the limit test carried out with 100 µg formulation./bee (equivalent to 85 µg a.s./bee). Corrected mortality after 48 hours was reported to be about 23% for oral and about 20% for contact exposure. Since calculated $HQ_{contact}$ and $ETR_{acute\ adult\ oral}$ for formulation are rather close to the relevant triggers and real LD_{50} is supposed to be much higher than 100 µg formulation./bee, it is considered acceptable to base the risk assessment on active substance toxicity data only.

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

Regarding the chronic adult risk for "treated crop" scenario, the Notifier provided the following justification: *"Considering that Dazide Enhance is a plant growth regulator that interferes with gibberellic acid biosynthesis to cause the plant to grow more "compacted" (by inhibition of intermodal elongation) and is applied by knapsack sprayer prior to flowering, the crop will not be attractive to foraging bees. …Daminozide is also not persistent in soil (maximum DT_{50} of 0.37 days) so residues are not expected to be taken up by plants at significant levels later in the growing season when flowers are present."* This is agreed by the RMS and the chronic risk to bees from the proposed use of daminozide is considered to be low.

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

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

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

[a] *As application is until BBCH 50 and no default value is available for ornamentals BBCH <50, a deposition factor of 60% is assumed, for plants with a similar structure (e.g. strawberries)*
[b] *Application after emergence of weeds*
[c] *Application before emergence of weed*
Ef: exposure factor
twa: time weighted average (default)

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

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

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

2) Protected use

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

Species	Test substance	Scenario	Risk quotient	HQ/ETR	Trigger
Screening level assessment					
Apis mellifera	a.s.	Not relevant	$HQ_{contact}$	<38.3	42
Apis mellifera	a.s.	Not relevant	$ETR_{acute\ adult\ oral}$	**<0.29**	0.2
Apis mellifera	Preparation	Not relevant	$HQ_{contact}$	**<50**	42
Apis mellifera	Preparation	Not relevant	$ETR_{acute\ adult\ oral}$	**<0.38**	0.2
Apis mellifera	a.s.	Not relevant	$ETR_{chronic\ adult\ oral}$	**<0.547**	0.03
Apis mellifera	a.s.	Not relevant	$ETR_{chronic\ larva\ oral}$	**0.34**	0.2
Tier 1 level assessment – BBCH <10 (leafy vegetables)					
Apis mellifera	a.s.	treated crop	$ETR_{acute\ adult\ oral}$	0.03	0.2
Apis mellifera	a.s.	treated crop	$ETR_{chronic\ adult\ oral}$	0.028	0.03
Apis mellifera	a.s.	treated crop	$ETR_{acute\ larva\ oral}$	0.03	0.2

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

Figures in bold exceed the relevant trigger value

Risk assessment for honeybees from consumption of contaminated water

Species	Test substance	Risk quotient	ETR	Trigger
Risk assessment from exposure to residues in guttation fluid (water solubility = 128 g/L)				
Apis mellifera	a.s.	$ETR_{acute\ adult\ oral}$	**7.3**	0.2
Apis mellifera	a.s.	$ETR_{chronic\ adult\ oral}$	**7.42**	0.03
Apis mellifera	a.s.	$ETR_{chronic\ larva\ oral}$	**102.3**	0.2

Species	Test substance	Risk quotient	ETR	Trigger
Risk assessment from exposure to residues in surface water (FOCUS step 2 PECsw of 0.1 mg/L)				
Apis mellifera	a.s.	$ETR_{acute\ adult\ oral}$	0.00	0.2
Apis mellifera	a.s.	$ETR_{chronic\ adult\ oral}$	0.000	0.03
Apis mellifera	a.s.	$ETR_{chronic\ larva\ oral}$	0.00	0.2
Risk assessment from exposure to residues in puddle water				
Apis mellifera	a.s.	$ETR_{acute\ adult\ oral}$	0.00	0.2
Apis mellifera	a.s.	$ETR_{chronic\ adult\ oral}$	0.000	0.03
Apis mellifera	a.s.	$ETR_{chronic\ larva\ oral}$	0.00	0.2

Figures in bold exceed the relevant trigger value

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

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

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

Risk assessment for bumlebees and solitary bees:

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

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

Conclusion – risk to bees

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

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

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

2.9.10.2 Risk assessment for non-target arthropods other than bees

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

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

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

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

In field-HQ = max. single application rate * MAF / LR_{50}

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

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

Table 83: In- and off-field exposure of daminozide applied to ornamentals

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

Table 84: In-field and off-field hazard quotients (HQs) for standard laboratory terrestrial arthropods from the proposed use of daminozide

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

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

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

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

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

Table 85: Refined non-target arthropod in-field risk assessment for *T. pyri for* protected use (other than permanent greenhouses)

Crop	Species	Appl. rate [kg a.s./ha]	MAF	$PER_{in-field}$ [g a.s./ha]	LR_{50}; ER_{50} [kg a.s./ha]	Risk acceptable?
Ornamental <50 cm in height	*Typhlodromus pyri*	7.65	3.0	22.95	> 8.50	**No**
Ornamental >50 cm in height	*Typhlodromus pyri*	7.65	3.0	22.95	> 8.50	**No**

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

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

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

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

2.9.10.3 Risk assessment for non-target soil meso- and macrofauna

2.9.10.3.1 Earthworms

Calculation of TER values

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

Table 86 : TER calculations for earthworms

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

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

2.9.10.3.2 Non-target soil meso- and macrofauna (other than earthworms)

No data were available.

2.9.10.4 Risk assessment for soil nitrogen transformation

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

2.9.10.5 Risk assessment for non-target plants

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

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

Table 87: Toxicity Exposure Ratios for terrestrial non-target plants exposed to daminozide (worst case - ornamentals >50 cm in height)

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

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

[b] ER_{50} is used to calculate the Toxicity Exposure Ratio

[c] Toxicity Exposure Ratio = ER_{50}/PER_{drift}

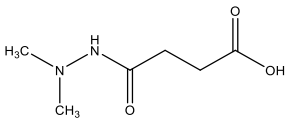
The calculated TER values, based on basic drift values for ornamentals >50 cm in height (worst-case) single application with a 3 meter buffer exceed the trigger of 5 in all species tested for effects on seedling emergence and vegetative vigour. This indicates that there will be negligible risk to non-target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

2.10 PROPOSED HARMONISED CLASSIFICATION AND LABELLING ACCORDING TO THE CLP CRITERIA [SECTIONS 1-6 OF THE CLH REPORT]

2.10.1 Identity of the substance [section 1 of the CLH report]

2.10.1.1 Name and other identifiers of the substance

Substance identity and information related to molecular and structural formula of the substance

Name(s) in the IUPAC nomenclature or other international chemical name(s)	daminozide (ISO); 4-(2,2-dimethylhydrazino)-4-oxobutanoic acid; *N*-dimethylaminosuccinamic acid
Other names (usual name, trade name, abbreviation)	Butanedioic acid mono(2,2-dimethylhydrazide)
ISO common name (if available and appropriate)	Daminozide (ISO); no synonyms
EC number (if available and appropriate)	-
EC name (if available and appropriate)	216-485-9
CAS number (if available)	1596-84-5
Other identity code (if available)	330 (CIPAC)
Molecular formula	$C_6H_{12}N_2O_3$
Structural formula	
SMILES notation (if available)	-
Molecular weight or molecular weight range	160.1711
Degree of purity (%) (if relevant for the entry in Annex VI)	*Min. 990 g/kg*

2.10.1.2 Composition of the substance

Constituents (non-confidential information)

Constituent (Name and numerical identifier)	**Concentration range (% w/w minimum and maximum in multi-constituent substances)**	**Current CLH in Annex VI Table 3.1 (CLP)**	**Current self- classification and labelling (CLP)**
N-dimethylaminosuccinamic acid or 4-(2,2-dimethylhydrazino)-4-oxobutanoic acid; Daminozide (ISO)	≥ 99% (w/w)		

Impurities (non-confidential information) if relevant for the classification of the substance

Impurity (Name and numerical identifier)	**Concentration range (% w/w minimum and maximum)**	**Current CLH in Annex VI Table 3.1 (CLP)**	**Current self-classification and labelling (CLP)**	**The impurity contributes to the classification and labelling**
N-nitrosodimethylamine (NDMA)	max 2.0 mg/kg	Carc. 1B Acute Tox. 2 * Acute Tox. 3 * STOT RE 1 Aquatic Chronic 2	Carc. 1B Acute Tox. 2 * Acute Tox. 3 * STOT RE 1 Aquatic Chronic 2	Yes
1,1-Dimethylhydrazide (UDMH)	max 30 mg/kg	Flam. Liq. 2 Carc. 1B Acute Tox. 3 * Acute Tox. 3 * Skin Corr. 1B Aquatic Chronic 2	Flam. Liq. 2 Carc. 1B Acute Tox. 3 * Acute Tox. 3 * Skin Corr. 1B Aquatic Chronic 2	Yes

Additives (non-confidential information) if relevant for the classification of the substance

Additive (Name and numerical identifier)	**Function**	**Concentration range (% w/w minimum and maximum)**	**Current CLH in Annex VI Table 3.1 (CLP)**	**Current self-classification and labelling (CLP)**	**The additive contributes to the classification and labelling**
-					

Test substances (non-confidential information)

Identification of test substance	**Purity**	**Impurities and additives (identity, %, classification if available)**	**Other information**	**The study(ies) in which the test substance is used**
Daminozide pure substance	99.9 % (w/w)			See tables of physical hazards
Daminozide technical substance	99.7 %(w/w)			See tables of physical hazards

2.10.2 Proposed harmonized classification and labelling

2.10.2.1 Proposed harmonised classification and labelling according to the CLP criteria

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	No current Annex VI entry										
Dossier submitters proposal	607-RST-VW-Y	daminozide (ISO); 4-(2,2-dimethylhydrazino)-4-oxobutanoic acid; *N*-dimethylaminosuccinamic acid	216-485-9	1596-84-5	Carc. 1B	H350	GHS08 Dgr	H350			
Resulting Annex VI entry if agreed by RAC and COM											

2.10.2.2 Additional hazard statements / labelling

Reason for not proposing harmonised classification and status under CLH public consultation

Hazard class	Reason for no classification	Within the scope of CLH public consultation
Explosives	data conclusive but not sufficient for classification	Yes
Flammable gases (including chemically unstable gases)	hazard class not applicable	No
Oxidising gases	hazard class not applicable	No
Gases under pressure	hazard class not applicable	No
Flammable liquids	hazard class not applicable	No
Flammable solids	data conclusive but not sufficient for classification	Yes
Self-reactive substances	hazard class not assessed in this dossier	No
Pyrophoric liquids	hazard class not applicable	No
Pyrophoric solids	hazard class not assessed in this dossier	No
Self-heating substances	data conclusive but not sufficient for classification	Yes
Substances which in contact with water emit flammable gases	hazard class not assessed in this dossier	No
Oxidising liquids	hazard class not applicable	No
Oxidising solids	data conclusive but not sufficient for classification	Yes
Organic peroxides	hazard class not applicable	No
Corrosive to metals	hazard class not assessed in this dossier	No
Acute toxicity via oral route	Conclusive, but not sufficient for classification	Yes
Acute toxicity via dermal route	Conclusive, but not sufficient for classification	Yes
Acute toxicity via inhalation route	Conclusive, but not sufficient for classification	Yes
Skin corrosion/irritation	Conclusive, but not sufficient for classification	Yes
Serious eye damage/eye irritation	Conclusive, but not sufficient for	Yes

Hazard class	Reason for no classification	Within the scope of CLH public consultation
	classification	
Respiratory sensitisation	Conclusive, but not sufficient for classification	Yes
Skin sensitisation	Conclusive, but not sufficient for classification	Yes
Germ cell mutagenicity	Conclusive, but not sufficient for classification	Yes
Carcinogenicity	-	Yes
Reproductive toxicity	Conclusive, but not sufficient for classification	Yes
Specific target organ toxicity-single exposure	Conclusive, but not sufficient for classification	Yes
Specific target organ toxicity-repeated exposure	Conclusive, but not sufficient for classification	Yes
Aspiration hazard	Hazard class not applicable	No
Hazardous to the aquatic environment	Conclusive but not sufficient for classification	Yes
Hazardous to the ozone layer	Conclusive, but not sufficient for classification	Yes

2.10.3 History of the previous classification and labelling

Not applicable. Daminozide has no previous classification and labelling.

2.10.4 Identified uses

Daminozide is used as a plant growth regulator. For more details, please refer to the GAP table under point 1.5

2.10.5 Data sources

Please refer to RAR Volumes 3 CA B1, B2, B6, B8 and B9.

2.11 Relevance of metabolites in groundwater

2.11.1 STEP 1: Exclusion of Degradation Products of No Concern

In soil, metabolite M1 (methanol) exceeded 10% AR in all four soils on more than one occasion. Methanol does not meet any of the conditions set out in the guidance document 'SANCO/221/2000 – rev.10- final, 25 February

2003' to be considered as a degradation product of no concern. Therefore, further consideration is necessary.

2.11.2 STEP 2: Quantification of Potential Groundwater Contamination

PECgw values for methanol were calculated using FOCUS groundwater scenarios and PEARL 4.4.4 model. The uses considered were 5 x 7.65 kg a.s./ha for indoor use on ornamentals and 5 x 4.2.5 kg a.s./ha for field use on ornamentals.

Predicted environmental concentrations for methanol where applications were made both indoors and in the field resulted in all scenarios displaying PECgw values <0.1 μg/L.

Therefore, no further consideration is required for methanol on the basis that the metabolite will not leach into groundwater al levels above 0.1 μg/L.

2.11.3 STEP 3: Hazard Assessment: Identification of relevant metabolites

2.11.3.1 STEP 3, STAGE 1: Screening for biological activity

No further assessment needed as methanol is not predicted to leach into groundwater at levels above 0.1 μg/L.

2.11.3.2 STEP 3, STAGE 2: Screening for genotoxicity

No further assessment needed as methanol is not predicted to leach into groundwater at levels above 0.1 μg/L.

2.11.3.3 STEP 3, STAGE 3: Screening for toxicity

No further assessment needed as methanol is not predicted to leach into groundwater at levels above 0.1 μg/L.

2.11.4 STEP 4: Exposure assessment – threshold of concern approach

No further assessment needed as methanol is not predicted to leach into groundwater at levels above 0.1 μg/L.

2.11.5 STEP 5: Refined risk assessment for non-relevance of metabolites

No further assessment needed as methanol is not predicted to leach into groundwater at levels above 0.1 μg/L.

2.11.6 Overall Conclusion

Methanol is not predicted to leach into groundwater at levels above 0.1 μg/L and hence no further evaluation of its biological activity or toxicity profile is necessary.

2.12 Consideration of isomeric composition in the risk assessment

Daminozide is not an isomeric compound. Further consideration of the isomeric composition in the risk assessment is therefore not required.

2.13 Residue Definitions

2.13.1 Definition of residues for exposure/risk assessment

Food of plant origin: Daminozide (sum of daminozide and 1,1-dimethyl-hydrazine (UDMH) expressed as daminozide)

Food of animal origin: Daminozide (sum of daminozide and 1,1-dimethyl-hydrazine (UDMH) expressed as

daminozide)

Soil: daminozide, methanol

Surface water: daminozide, methanol

Sediment: daminozide, methanol

Ground water: daminozide, methanol

Air: daminozide, methanol

2.13.2 Definition of residues for monitoring

Food of plant origin: Daminozide (sum of daminozide and 1,1-dimethyl-hydrazine (UDMH) expressed as daminozide)

Food of animal origin: Daminozide (sum of daminozide and 1,1-dimethyl-hydrazine (UDMH) expressed as daminozide)

Soil: daminozide

Surface water: daminozide

Sediment: daminozide

Ground water: daminozide

Air: daminozide, UDMH

LEVEL 3

3 PROPOSED DECISION WITH RESPECT TO THE APPLICATION

3.1 Background to the proposed decision

3.1.1 Proposal on acceptability against the approval criteria – Article 4 and Annex II of Regulation (EC) No 1107/2009

3.1.1.1 Article 4		**Yes**	**No**	
	It is considered that Article 4 of Regulation (EC) No 1107/2009 is complied with. Specifically the RMS considers that authorisation in at least one Member State is expected to be possible for at least one plant protection product containing the active substance for at least one of the representative uses.			This will depend on the outcome of the expert discussion on carcinogenicity.
3.1.1.2 Submission of further information (Annex II 2.2)		**Yes**	**No**	
i)	It is considered that a complete dossier has been submitted		X	See point 3.1.5 – Issues that could not be finalized
ii)	It is considered that in the absence of a full dossier the active substance may be approved even though certain information is still to be submitted because: (a) the data requirements have been amended or refined after the submission of the dossier; or (b) the information is considered to be confirmatory in nature, as required to increase confidence in the decision.	X		
3.1.1.3 Restrictions on approval (Annex II 2.3)		**Yes**	**No**	
	It is considered that in line with Article 6 of Regulation (EC) No 1107/2009 approval should be subject to conditions and restrictions.		X	
3.1.1.4 Criteria for the approval of an active substance (Annex II 3)				
Dossier (Annex II 3.1)		**Yes**	**No**	
i)	It is considered the dossier contains the information needed to establish, where relevant, Acceptable Daily Intake (ADI), Acceptable Operator Exposure Level (AOEL) and Acute Reference Dose (ARfD).	X		The provided data are sufficient for establishing reference values.
ii)	It is considered that the dossier contains the	X		

	information necessary to carry out a risk assessment and for enforcement purposes (relevant for substances for which one or more representative uses includes use on feed or food crops or leads indirectly to residues in food or feed). In particular it is considered that the dossier: (a) permits any residue of concern to be defined; (b) reliably predicts the residues in food and feed, including succeeding crops (c) reliably predicts, where relevant, the corresponding residue level reflecting the effects of processing and/or mixing; (d) permits a maximum residue level to be defined and to be determined by appropriate methods in general use for the commodity and, where appropriate, for products of animal origin where the commodity or parts of it is fed to animals; (e) permits, where relevant, concentration or dilution factors due to processing and/or mixing to be defined.			
iii)	It is considered that the dossier submitted is sufficient to permit, where relevant, an estimate of the fate and distribution of the active substance in the environment, and its impact on non-target species.	X		
Efficacy (Annex II 3.2)		**Yes**	**No**	
	It is considered that it has been established for one or more representative uses that the plant protection product, consequent on application consistent with good plant protection practice and having regard to realistic conditions of use is sufficiently effective.	X		
Relevance of metabolites (Annex II 3.3)		**Yes**	**No**	
	It is considered that the documentation submitted is sufficient to permit the establishment of the toxicological, ecotoxicological or environmental relevance of metabolites.	X		
Composition (Annex II 3.4)		**Yes**	**No**	
i)	It is considered that the specification defines the minimum degree of purity, the identity and	X		Sufficient information has been presented to support the declared technical specification of daminozide with

	maximum content of impurities and, where relevant, of isomers/diastereo-isomers and additives, and the content of impurities of toxicological, ecotoxicological or environmental concern within acceptable limits.			respect to the identity and content of impurities in the respective technical specifications. The minimum purity of daminozide should be specified as 990 g/kg
ii)	It is considered that the specification is in compliance with the relevant Food and Agriculture Organisation specification, where such specification exists.			Not relevant (no FAO specification available for daminozide)
iii)	It is considered for reasons of protection of human or animal health or the environment, stricter specifications than that provided for by the FAO specification should be adopted.			Not relevant (see above)
Methods of analysis (Annex II 3.5)		**Yes**	**No**	
i)	It is considered that the methods of analysis of the active substance, safener or synergist as manufactured and of determination of impurities of toxicological, ecotoxicological or environmental concern or which are present in quantities greater than 1 g/kg in the active substance, safener or synergist as manufactured, have been validated and shown to be sufficiently specific, correctly calibrated, accurate and precise.	X		Adequate analytical methods are available for the determination of daminozide and all significant and relevant impurities in the technical material.
ii)	It is considered that the methods of residue analysis for the active substance and relevant metabolites in plant, animal and environmental matrices and drinking water, as appropriate, shall have been validated and shown to be sufficiently sensitive with respect to the levels of concern.		X	Adequate methods are available to monitor the respective current residue definition in soil, drinking water, surface water and air. Method for air (daminozide) is available, but validation is not sufficient (LOQ is not low enough). Methods for the determination of daminozide residues in or on food and feed of plant and animal origin are not required. Methods for the determination of daminozide residues in body fluids and tissues are required.
iii)	It is confirmed that the evaluation has been carried out in accordance with the uniform principles for evaluation and authorisation of plant protection products referred to in Article 29(6) of Regulation 1107/2009.	X		The information submitted with regards to methods of analysis is sufficient to support approval.
Impact on human health (Annex II 3.6)				
Impact on human health - ADI, AOEL, ARfD (Annex II 3.6.1)		**Yes**	**No**	

	It is confirmed that (where relevant) an ADI, AOEL and ARfD can be established with an appropriate safety margin of at least 100 taking into account the type and severity of effects and the vulnerability of specific groups of the population.	X		The calculation of the ADI is based on the results of a two-year carcinogenicity study in rat. The increased incidence of pituitary adenomas was observed at females already from the lowest dose, therefore a provisional "NOAEL" of 5 mg/kg bw/day has been established. Application of an assessment factor of 100 and additional safety factor of 2 resulted in ADI of 0.025 mg/kg bw/day Due to the frequent use pattern of formulations based on daminozide, the AOEL is based on the results of two-year carcinogenicity study in rat. The provisional "NOAEL" from long-term studies was 5 mg/kg bw/day. By using a safety factor of 100, additional safety factor of 2 and adjustment for 35% oral absorption, this results in a long-term systemic AOEL of 0.009 mg/kg bw/day. ARFD was not established
Impact on human health – proposed genotoxicity classification (Annex II 3.6.2)		**Yes**	**No**	
	It is considered that, on the basis of assessment of higher tier genotoxicity testing carried out in accordance with the data requirements and other available data and information, including a review of the scientific literature, reviewed by the Authority, **the substance SHOULD BE classified or proposed for classification**, in accordance with the provisions of Regulation (EC) No 1272/2008, **as mutagen category 1A or 1B**.		X	Daminozide - Based on the negative results of *in vitro* and new *in vivo* studies, daminozide is considered to have no genotoxic properties. UDMH is main metabolite and impurity of daminozide. Based on the available data, the genotoxic potential of UDMH cannot be unequivocally concluded – for further details see point 2.6.8
Impact on human health – proposed carcinogenicity classification (Annex II 3.6.3)		**Yes**	**No**	
i)	It is considered that, on the basis of assessment of the carcinogenicity testing carried out in accordance with the data requirements for the active substances, safener or synergist and other available data and information, including a review of the scientific literature, reviewed by the Authority, **the substance SHOULD BE classified or proposed for classification**, in accordance with the provisions of Regulation (EC) No 1272/2008, **as carcinogen category 1A or 1B**.	X		For further details see point 2.6.5 for daminozide and point 2.6.8 for UDMH.

ii)	Linked to above classification proposal. It is considered that exposure of humans to the active substance, safener or synergist in a plant protection product, under realistic proposed conditions of use, is negligible, that is, the product is used in closed systems or in other conditions excluding contact with humans and where residues of the active substance, safener or synergist concerned on food and feed do not exceed the default value set in accordance with Article 18(1)(b) of Regulation (EC) No 396/2005.		X	
Impact on human health – proposed reproductive toxicity classification (Annex II 3.6.4)		**Yes**	**No**	
i)	It is considered that, on the basis of assessment of the reproductive toxicity testing carried out in accordance with the data requirements for the active substances, safeners or synergists and other available data and information, including a review of the scientific literature, reviewed by the Authority, **the substance SHOULD BE classified or proposed for classification**, in accordance with the provisions of Regulation (EC) No 1272/2008, **as toxic for reproduction category 1A or 1B**.		X	There were no effects observed in relevant studies.
ii)	Linked to above classification proposal. It is considered that exposure of humans to the active substance, safener or synergist in a plant protection product, under realistic proposed conditions of use, is negligible, that is, the product is used in closed systems or in other conditions excluding contact with humans and where residues of the active substance, safener or synergist concerned on food and feed do not exceed the default value set in accordance with Article 18(1)(b) of Regulation (EC) No 396/2005.			Not applicable
Impact on human health – proposed endocrine disrupting properties classification (Annex II 3.6.5)		**Yes**	**No**	
i)	It is considered that **the substance SHOULD BE classified or proposed for classification** in accordance with the provisions of Regulation (EC) No 1272/2008, **as carcinogenic category 2**		X	No effects with regard to the endocrine disruption were observed

	and toxic for reproduction category 2 and on that basis shall be considered to have endocrine disrupting properties.			
ii)	It is considered that **the substance SHOULD BE classified or proposed for classification** in accordance with the provisions of Regulation (EC) No 1272/2008, **as toxic for reproduction category 2 and** in addition the RMS considers the substance **has toxic effects on the endocrine organs and on that basis shall be considered to have endocrine disrupting properties**.		X	There were no effects observed in relevant studies.
iii)	Linked to either i) or ii) immediately above. It is considered that exposure of humans to the active substance, safener or synergist in a plant protection product, under realistic proposed conditions of use, is negligible, that is, the product is used in closed systems or in other conditions excluding contact with humans and where residues of the active substance, safener or synergist concerned on food and feed do not exceed the default value set in accordance with Article 18(1)(b) of Regulation (EC) No 396/2005.			
Fate and behaviour in the environment				
Persistent organic pollutant (POP) (Annex II 3.7.1)		**Yes**	**No**	
	It is considered that the active substance **FULFILS** the criteria of a persistent organic pollutant (POP) as laid out in Regulation 1107/2009 Annex II Section 3.7.1.			Normalised laboratory soil DT50 to 12°C = 0.1 – 0.5 days Normalised whole system water/sediment DT50 to 12°C = 1.9 – 2.0 days No reliable DT50 in surface water, **data gap**
Persistent, bioaccumulative and toxic substance (PBT) (Annex II 3.7.2)		**Yes**	**No**	
	It is considered that the active substance **FULFILS** the criteria of a persistent, bioaccumulative and toxic (PBT) substance as laid out in Regulation 1107/2009 Annex II Section 3.7.2.			Normalised laboratory soil DT50 to 12°C = 0.1 – 0.5 days Normalised whole system water/sediment DT50 to 12°C = 1.9 – 2.0 days No reliable DT50 in surface water, **data gap** Based on the proposed classification (Carc. 1B) the substance fullfils criteria for toxicity.
Very persistent and very bioaccumulative substance (vPvB) (Annex II 3.7.3)		**Yes**	**No**	
	It is considered that the active substance **FULFILS** the criteria of a a very persistent and			Normalised laboratory soil DT50 to 12°C = 0.1 – 0.5 days

	very bioaccumulative substance (vPvB) as laid out in Regulation 1107/2009 Annex II Section 3.7.3.			Normalised whole system water/sediment DT50 to 12°C = 1.9 – 2.0 days No reliable DT50 in surface water, **data gap**
Ecotoxicology (Annex II 3.8)		**Yes**	**No**	
i)	It is considered that the risk assessment demonstrates risks to be acceptable in accordance with the criteria laid down in the uniform principles for evaluation and authorisation of plant protection products referred to in Article 29(6) under realistic proposed conditions of use of a plant protection product containing the active substance, safener or synergist. The RMS is content that the assessment takes into account the severity of effects, the uncertainty of the data, and the number of organism groups which the active substance, safener or synergist is expected to affect adversely by the intended use.		X	No acute risks were identified for birds for field use. No acute and reproductive risks were identified for birds for protected use in permanent greenhouses. High dietary reproductive risk was concluded for small insectivorous bird (blue tit) for field use. High dietary acute and reproductive risk was concluded for small insectivorous bird (blue tit) for protected use other than permanent greenhouses. No acute risks were identified for mammals for field use. No acute and reproductive risks were identified for birds for protected use in permanent greenhouses. High dietary reproductive risk was concluded for small herbivorous mammal scenario (common vole) for field use. High dietary acute and reproductive risk was concluded for small herbivorous mammal scenario (common vole) for protected use other than permanent greenhouses. No acute risks were identified for fish and aquatic invertebrates and no chronic risks were identified for fish and algae from daminozide and its metabolite methanol. No valid chronic toxicity data for aquatic invertebrates and aquatic macrophytes were available, neither for daminozide nor for methanol. Therefore, no chronic risk assessment could be performed for aquatic invertebrates and aquatic macrophytes. Thus, aquatic risk assessment for both daminozide and methanol could not be finalized. No risks were identified for bees for field use and protected use other than permanent greenhouses (when relevant mitigation measures are considered), except for consumption of guttation fluid where high risk was concluded. No risks were identified for bees for protected use in permanent greenhouses (when relevant mitigation measures are considered). No risks were identified for non-target arthropods other

				than bees for field use and protected use in permanent greenhouses. High risk to non-target arthropods was identified for protected uses other than permanent greenhouses. The risk to beneficial arthropods, used in Integrated Pest Management (IPM) in permanent grrenhouses, is considered to be low, while for protected uses other than permanent greenhouses is considered high. No risks were identified for earthworms. Since no valid endpoint for soil nitrogen transformation was available no risk assessment could be performed. Thus, risk assessment for soil microorganisms could not be finalized. No risks were identified for non-target flora. For further information on risks to non-target flora and fauna, see Vol 1 Level 2.6.
ii)	It is considered that, on the basis of the assessment of Community or internationally agreed test guidelines, the substance **HAS** endocrine disrupting properties that may cause adverse effects on non-target organisms.		X	
iii)	Linked to the consideration of the endocrine properties immediately above. It is considered that the exposure of non-target organisms to the active substance in a plant protection product under realistic proposed conditions of use is negligible.			
iv)	It is considered that it is established following an appropriate risk assessment on the basis of Community or internationally agreed test guidelines, that the use under the proposed conditions of use of plant protection products containing this active substance, safener or synergist: — will result in a negligible exposure of honeybees, or — has no unacceptable acute or chronic effects on colony			No risks were identified for bees for field use and protected use other than permanent greenhouses (when relevant mitigation measures are considered), except for consumption of guttation fluid where high risk was concluded. No risks were identified for bees for protected use in permanent greenhouses (when relevant mitigation measures are considered). The risk assessment for bees should be discussed in peer review.

	survival and development, taking into account effects on honeybee larvae and honeybee behaviour.			
Residue definition (Annex II 3.9)		**Yes**	**No**	
	It is considered that, where relevant, a residue definition can be established for the purposes of risk assessment and for enforcement purposes.	X		<u>Definition of residues for exposure/risk assessment:</u> Food of plant origin: Daminozide (sum of daminozide and 1,1-dimethyl-hydrazine (UDMH) expressed as daminozide) Food of animal origin: Daminozide (sum of daminozide and 1,1-dimethyl-hydrazine (UDMH), expressed as daminozide) Soil: daminozide, methanol Surface water: daminozide, methanol Ground water: daminozide, methanol Air: daminozide, methanol EFATE residue definition is provisional <u>Definition of residues for monitoring</u> Food of plant origine: Daminozide (sum of daminozide and 1,1-dimethyl-hydrazine (UDMH), expressed as daminozide) Food of animal origin: Daminozide (sum of daminozide and 1,1-dimethyl-hydrazine (UDMH), expressed as daminozide) Soil: daminozide Surface water: daminozide Sediment: daminozide Ground water: daminozide Air: daminozide, UDMH
Fate and behaviour concerning groundwater (Annex II 3.10)		**Yes**	**No**	
	It is considered that it has been established for one or more representative uses, that consequently after application of the plant protection product consistent with realistic conditions on use, the predicted concentration of the active substance or of metabolites, degradation or reaction products in groundwater complies with the respective criteria of the uniform principles for evaluation and authorisation of plant protection products referred to in Article 29(6) of Regulation 1107/2009.	X		PECgw values for daminozide were <<0.1 µg/L in all scenarios, for applications made both in the field and in glasshouses. For methanol, applications made both indoors and in the field resulted in all scenarios displaying PECgw values <0.1 µg/L. A relevance assessment for methanol is therefore not required.

3.1.2 Proposal - Candidate for substitution

Candidate for substitution		Yes	No	
	It is considered that the active substance shall be approved as a candidate for substitution	X		Based on the proposed classification Carc 1B., daminozide is considered to be a candidate for substition.

3.1.3 Proposal – Low risk active substance

Low-risk active substances		Yes	No	
	It is considered that the active substance **shall be considered of low risk**. In particular it is considered that the substance **should NOT be classified or proposed for classification** in accordance with Regulation (EC) No 1272/2008 as at least one of the following: — carcinogenic, — mutagenic, — toxic to reproduction, — sensitising chemicals, — very toxic or toxic, — explosive, — corrosive. In addition, it is considered that **the substance is NOT**: — persistent (half-life in soil more than 60 days), — has a bioconcentration factor higher than 100, — is deemed to be an endocrine disrupter, or — has neurotoxic or immunotoxic effects.		X	

3.1.4 List of studies to be generated, still ongoing or available but not evaluated

Data gap	Relevance in relation to representative use(s)	Study status		
		No confirmation that study available or on-going	Study on-going and anticipated date of completion	Study available but not peer-reviewed
3.1.4.1 Identity of the active substance or formulation(s)				
none				
3.1.4.2 Physical and chemical properties of the active substance and physical, chemical and technical properties of the formulation(s)				
none				
3.1.4.3 Data on uses and efficacy				
none				
3.1.4.4 Data on handling, storage, transport, packaging and labelling				
none				
3.1.4.5 Methods of analysis				
Air	Validation of the method (for daminozide) is not	x		

	sufficient.			
Body fluids and tissues	It is required according to Commission Regulation (EU) No 283/2013.		x (Q4 2018)	
3.1.4.6 Toxicology and metabolism				
none				
3.1.4.7 Residue data				
none				
3.1.4.8 Environmental fate and behaviour				
New Adsorption/desorption study for daminozide	All			x
Short-range and long-range transport in air for methanol	All	x		
3.1.4.9 Ecotoxicology				
Chronic toxicity study on Daphnia. Toxicity study on aquatic macrophyte. Study on effects on soil nitrogen transformation.	All			x

3.1.5 Issues that could not be finalised

Area of the risk assessment that could not be finalised on the basis of the available data[1)]		**Relevance in relation to representative use(s)**
TOX	Genotoxic properties of the main metabolite and impurity UDMH cannot be concluded.	All
TOX	The role of UDMH in human metabolism cannot be concluded.	All
ECOTOX	No chronic risk assessment could be performed for aquatic invertebrates and aquatic macrophytes since no valid data were available.	All
ECOTOX	No risk assessment for effects on soil nitrogen transformation could be performed since no valid data were available.	All
EFATE	Short-range and long-range transport in air for methanol	All (ornamental crops grown in the field and indoor)
EFATE	Effect of water treatment processes on the nature of residues present in surface and groundwater, when surface water or groundwater are abstracted for drinking water.	All (ornamental crops grown in the field and indoor)

1) An issue is listed as an issue that could not be finalised where there is not enough information available to perform an

assessment, even at the lowest tier level, for the representative uses in line with the Uniform Principles, as laid out in Commission Regulation (EU) No 546/2011, and where the issue is of such importance that it could, when finalised, become a concern (which would also be listed as a critical area of concern if it is of relevance to all representative uses).

3.1.6 Critical areas of concern

Critical area of concern identified[1)]		**Relevance in relation to representative use(s)**
1)	Proposed classification Carc. 1B	ALL

1) An issue is listed as a critical area of concern:
(a) where the substance does not satisfy the criteria set out in points 3.6.3, 3.6.4, 3.6.5 or 3.8.2 of Annex II of Regulation (EC) No 1107/2009 and the applicant has not provided detailed evidence that the active substance is necessary to control a serious danger to plant health which cannot be contained by other available means including non-chemical methods, taking into account risk mitigation measures to ensure that exposure of humans and the environment is minimised, or
(b) where there is enough information available to perform an assessment for the representative uses in line with the Uniform Principles, as laid out in Commission Regulation (EU) 546/2011, and where this assessment does not permit to conclude that for at least one of the representative uses it may be expected that a plant protection product containing the active substance will not have any harmful effect on human or animal health or on groundwater or any unacceptable influence on the environment.
An issue is also listed as a critical area of concern where the assessment at a higher tier level could not be finalised due to a lack of information, and where the assessment performed at the lower tier level does not permit to conclude that for at least one of the representative uses it may be expected that a plant protection product containing the active substance will not have any harmful effect on human or animal health or on groundwater or any unacceptable influence on the environment.

3.1.7 Overview table of the concerns identified for each representative use considered

Representative use:		**Ornamental crops**	
		Field use	**Indoor use**
Operator risk	Risk identified	X	X
	Assessment not finalised	X	X
Worker risk	Risk identified	-	X
	Assessment not finalised	-	X
Bystander risk	Risk identified	X	-
	Assessment not finalised	X	-
Consumer risk	Risk identified	-	-
	Assessment not finalised	-	-
Risk to wild non target terrestrial vertebrates	Risk identified	X	X
	Assessment not finalised	X	X
Risk to wild non target terrestrial organisms other than vertebrates	Risk identified	X	X
	Assessment not finalised	X	X
Risk to aquatic organisms	Risk identified	X	X
	Assessment not finalised	X	X
Groundwater exposure active substance	Legal parametric value breached	-	-
	Assessment not finalised	-	-
Groundwater exposure metabolites	Legal parametric value breached	-	-
	Parametric value of 10 µg/L[(a)] breached	-	-
	Assessment not finalised	-	-
Comments/Remarks			

The superscript numbers in this table relate to the numbered points indicated within chapter 3.1.5 and 3.1.6. Where there is no superscript number, see level 2 for more explanation.
(a) Value for non relevant metabolites prescribed in SANCO/221/2000-rev 10-final, European Commission, 2003

3.1.8 Area(s) where expert consultation is considered necessary

It is recommended to organise a consultation of experts on the following parts of the assessment report:

Area(s) where expert consultation is considered necessary	Justification
ECOTOX – Reproductive dietary risk assessment for wild mammals.	The selection of ecotoxicologically relevant endpoint to be used in the reproductive risk assessment for wild mammals needs to be discussed in peer review.
ECOTOX – Risk assessment for aquatic organisms from metabolite methanol	
ECOTOX – Risk assessment for bees	No scenario for ornamentals is included in the EFSA Guidance (2013), therefore, a surrogate scenario for leafy vegetables has been used by RMS.
TOX	Carcinogenic properties of Daminozide and UDMH need to be discussed on the expert meeting.

3.1.9 Critical issues on which the Co-RMS did not agree with the assessment by the RMS

Points on which the co-rapporteur Member State did not agree with the assessment by the rapporteur member state. Only the points relevant for the decision making process are listed.

Issue on which Co-RMS disagrees with RMS	Opinion of Co-RMS	Opinion of RMS

3.2 Proposed decision

It is proposed that:

Daminozide cannot be approved under Regulation (EC) No 1107/2009 because of the:

1) Proposed classification as Carc 1B.
2) Genotoxic properties of the main metabolite and impurity UDMH cannot be concluded based on the submitted data.

3.3 Rationale for the conditions and restrictions to be associated with any approval or authorisation(s), as appropriate

-

3.3.1 Particular conditions proposed to be take into account to manage the risks identified

Proposed condition/risk mitigation measure	Relevance in relation to representative use(s)
-	

ANNEX 1 – BACKGROUND DOCUMENT TO RAC OPINION ON DAMINOZIDE (ISO); 4-(2,2-DIMETHYLHYDRAZINO)-4-OXOBUTANOIC ACID; N-DIMETHYLAMINOSUCCINAMIC ACID

APPENDICES

Appendix 1: Guidance documents used in this assessment

European Commission, 2012. Guidance Document on the Assessment of the Equivalence of Technical Materials of Substances Regulated under Council Directive 91/414/EEC (SANCO/10597/2003 – rev. 10)

European Commission, 2000. Residues: Guidance for generating and reporting methods of analysis in support of pre-registration data requirements for Annex II (part A, Section 4) and Annex III (part A, Section 5) of Directive 91/414 (SANCO/3029/99 rev. 4)

European Commission, 2000. Technical Material and Preparations: Guidance for generating and reporting methods of analysis in support of pre- and post-registration data requirements for Annex II (part A, Section 4) and Annex III (part A, Section 5) of Directive 91/414 (SANCO/3030/99 rev. 4)

European Commission, 2010. Guidance document on pesticide residue analytical methods (SANCO/825/00 rev. 8.1)

OECD Test guideline 401: Acute oral Toxicity

OECG Test guideline 402: Acute Dermal Toxicity

OECD Test guideline 403: Acute Inhalation toxicity

OECD Test Guideline 404: Acute Dermal Irritaton/Corrosion

OECD Test Guideline 405: AcuteEye Irritation/Corrosion

OECD Test Guideline 406: Skin Sensitisatin

OECD Test Guideline 408: Subchronic Oral Toxicity – Rodent: 90 day Study

OECD Test Guideline 409: Subchronic Oral Toxicity – Non-rodent: 90 day Study

OECD Test Guideline 412: Repeated Dose Inhalation Toxicity: 28-day or 14-day study

OECD Test Guideline 410: Repeated Dermal Toxicity 21/28 day Study

OECD Test Guideline 414: Teratogenicity

OECD Test Guideline 416: Two-Generation Reproduction Toxicity

OECD Test Guideline 417: Toxicokinetcs

OECD Test Guideline 424: Neurotoxicity Study In Rodents

OECD Test Guideline 425: Acute Oral Toxicity – Up-and-Down Procedure

OECD Test Guideline 451 Carcinogenicity studies

OECD Test Guideline 453: Combined Chronic Toxicity/Carcinogenicity Studies

OECD Test Guideline 471: Genetic Toxicology: Salmonella typhimurium, Reverse mutation Assay

OECD Test Guideline 472: Genetic Toxicology: Escherichia coli, Reverse mutation Assay

OECD Test Guideline 473: Genetic Toxicology: *In vitro* Mammalian Cytogenetic Test

OECD Test Guideline 474 Genetic Toxicology: Micronucleus test

OECD Test Guideline 476: Genetic Toxicology: *In vitro* Mammalian Cell Gene Mutation Tests

OECD Test Guideline 486: Unscheduled DNA Synthesis (UDS) Test with Mammalian Liver Cells in vivo

OPPTS 870.7800: Immunotoxicity

The EFSA Journal, 2012. Guidance on Dermal Absorption. 10(4): 2665.

OECD Test Guideline 501: Metabolism in crops

OECD Test Guideline 502: Metabolism in rotational crops.

OECD Test Guideline 503: Metabolism in livestock

OECD Test Guideline 504: Residues in rotational crops (limited field studies).

OECD Test Guideline 505: Residues in livestock.

OECD Test Guideline 506: Stability of pesticide residues in stored commodities

OECD Test Guideline 507: Nature of the pesticide residues in processed commodities – High temperature hydrolysis.

OECD Test Guideline 508: Magnitude of the pesticide residues in processed commodities.

OECD Test Guideline 509: Crop field trials

OECD (2009). Guidance Document on the Definition of Residues. Environment, Health and Safety Publications. Series on Testing and Assessment No. 63 and Series on Pesticides No. 31

OECD (2009). Guidance Document on Overview of Residue Chemistry Studies (as revised in 2009). Environment, Health and Safety Publications. Series on Testing and Assessment No. 64 and Series on Pesticides No. 32.

OECD (2011) Guidance Document on Crop Field Trials (Series on Testing and Assessment No. 164 and Series on Pesticides No. 66)

OECD (2013) Guidance Document on residues in livestock (Series on Pesticides No. 73)

OECD (2008). Guidance document on magnitude of pesticide residues in processed commodities. Environment, Health and Safety Publications. Series on Testing and Assessment No. 96.

Revision 2.0 of the EFSA model. Reasoned Opinion on the Potential Chronic and Acute Risk to Consumers' Health Arising from Proposed Temporary EU MRLs According to Regulation (EC) No 396/2005 on Maximum Residue Levels of Pesticides in Food and Feed of Plant and Animal Origin, European Food Safety Authority, 15 March 2007

Predictive Operator Exposure Model (POEM), UK MAFF, 1992, (revised 2007).

Uniform Principles for Safeguarding the Health of Applicators of Plant Protection Products (Uniform Principles for Operator Protection): Mitteilungen aus der Biologischen Bundesanstalt fur Land- und Forstwirtschaft, Berlin-Dahlem, no. 277, 1992.

S. Martin, D. Westphal, M. Erdtmann-Vourliotis, F. Dechet, C. Schulze-Rosario, F. Stauber, H. Wicke and G. Chester, "Guidance for Exposure and Risk Evaluation for Bystanders and Residents Exposed to Plant Protection Products During and After Application," Journal of Consumer Protection and Food Safety 3 (2008) 272-281.

Bystander Exposure Guidance, UK CRD Regulatory Update 10/2008, April 22, 2008
European Commission, 2003. Guidance Document on Assessment of the Relevance of Metabolites in Groundwater of Substances Regulated under Council Directive 91/414/EEC. SANCO/221/2000-rev. 10 - final, 25 February 2003.

FOCUS (1996). Soil Persistence Models and EU Registration, European Commission Document No. 7617/VI/96.
FOCUS, 2000. "FOCUS Groundwater Scenarios in the EU review of active substances". Report of the FOCUS Groundwater Scenarios Workgroup, EC Document Reference SANCO/321/2000-rev.2. 202 pp, as updated by the Generic Guidance for FOCUS groundwater scenarios, version 1.1 dated April 2002.

FOCUS, 2001. "FOCUS Surface Water Scenarios in the EU Evaluation Process under 91/414/EEC". Report of the FOCUS Working Group on Surface Water Scenarios, EC Document Reference SANCO/4802/2001-rev.2. 245 pp., as updated by the Generic Guidance for FOCUS surface water scenarios, version 1.1 dated March 2012

FOCUS, 2006. "Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration" Report of the FOCUS Work Group on Degradation Kinetics, EC Document Reference Sanco/10058/2005 version 2.0, 434 pp.

FOCUS, 2007. "Landscape And Mitigation Factors In Aquatic Risk Assessment. Volume 1. Extended Summary and Recommendations". Report of the FOCUS Working Group on Landscape and Mitigation Factors in Ecological Risk Assessment, EC Document Reference SANCO/10422/2005 v2.0. 169 pp.

FOCUS, 2009. "Assessing Potential for Movement of Active Substances and their Metabolites to Ground Water in the EU". Report of the FOCUS Workgroup, EC Document Reference SANCO/13144/2010-version.1. 604 pp, as outlined in Generic Guidance for Tier 1 FOCUS groundwater Assessment, version 2.0 dated January 2011.

FOCUS (2011) "Generic guidance for Tier 1 FOCUS Ground water scenarios". Version 2.0. FOCUS Version Control Group, European Commission.

FOCUS (2011) "Generic guidance for FOCUS Surface water scenarios". Version 1.0. FOCUS Version Control Group, European Commission.

FOCUS, 2011. Generic guidance for Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration". Ver 1, 23 November, 2011. FOCUS Version Control Group, European Commission.

FOCUS (2008). "Pesticides in Air: Considerations for Exposure Assessment". Report of the FOCUS Working Group on Pesticides in Air, EC Document Reference SANCO/10553/2006 Rev 2 June 2008. 327 pp.

EFSA (European Food Safety Authority), 2009. Guidance Document on Risk Assessment for Birds and Mammals on request of EFSA. EFSA Journal 2009; 7(12):1438.

European Commission, 2002a. Guidance Document on Terrestrial Ecotoxicology Under Council Directive 91/414/EEC. SANCO/10329/2002 rev.2 final, 17 October 2002.

European Commission, 2002b. Guidance Document on Aquatic Ecotoxicology Under Council Directive 91/414/EEC. SANCO/3268/2001 rev 4 (final), 17 October 2002.

European Commission, 2002c. Guidance Document on Risk Assessment for Birds and Mammals Under Council Directive 91/414/EEC. SANCO/4145/2000.

European Commission, 2003. Guidance Document on Assessment of the Relevance of Metabolites in Groundwater of Substances Regulated under Council Directive 91/414/EEC. SANCO/221/2000-rev. 10 - final, 25 February 2003.

SETAC (Society of Environmental Toxicology and Chemistry), 2001. Guidance Document on Regulatory Testing and Risk Assessment procedures for Plant Protection Products with Non-Target Arthropods. ESCORT 2.

The EFSA Journal, 2005. Opinion of the Scientific Panel on Plant health, Plant protection products and their Residues on a request from EFSA related to the assessment of the acute and chronic risk to aquatic organisms with regard to the possibility of lowering the uncertainty factor if additional species were tested. 301, 1-45.

European Food Safety Authority, 2013. EFSA Guidance Document on the risk assessment of plant protection products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees). EFSA Journal 2013;11(7):3295, 268 pp., doi:10.2903/j.efsa.2013.3295

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