Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

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

Adopted

11 June 2020

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON DAMINOZIDE (ISO); 4-(2,2-DIMETHYLHYDRAZINO)-4-OXOBUTANOIC ACID; *N*-DIMETHYLAMINOSUCCINAMIC ACID

COMMENTS AND RESPONSE TO COMMENTS ON CLH: PROPOSAL AND JUSTIFICATION

Comments provided during public consultation are made available in the table below as submitted through the web form. Any attachments received are referred to in this table and listed underneath, or have been copied directly into the table.

All comments and attachments including confidential information received during the public consultation have been provided in full to the dossier submitter (Member State Competent Authority), the Committees and to the European Commission. Non-confidential attachments that have not been copied into the table directly are published after the public consultation and are also published together with the opinion (after adoption) on ECHA's website. Dossier submitters who are manufacturers, importers or downstream users, will only receive the comments and non-confidential attachments, and not the confidential information received from other parties.

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Substance name: daminozide (ISO); 4-(2,2-dimethylhydrazino)-4-oxobutanoic acid; *N*-dimethylaminosuccinamic acid
EC number: 216-485-9
CAS number: 1596-84-5
Dossier submitter: Czech Republic

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
16.09.2019	United Kingdom	Daminozide Task Force	Company-Manufacturer	1
Comment received				
Attachement : Applicant supports and recommends to consider the "Opinion of the Scientific Panel on Plant Health, Plant Protection Products and their Residues on a request from the Commission related to the evaluation of daminozide in the context of Council Directive 91/414/EEC, EFSA Journal (2004), 61, 1-27." As a result PPR opinion Daminozide 2004 is attached for convenience. ECHA note – An attachment was submitted with the comment above. Refer to public attachment PPR opinion - daminozide 2004.pdf				
Dossier Submitter's Response				
Noted.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
24.09.2019	Germany		MemberState	2
Comment received				
The decomposition temperature is stated as 142 – 145 °C. As the melting point of 153 – 154.5 °C is higher as the decomposition temperature it is questionable whether this specified melting point corresponds to Daminozide and not to the products of the de-composition. For the endpoints relative density, viscosity and granulometry it is stated that they are not a requirement according to 283/2014. This is a typo as the correct regulation is 284/2013.				

Toxicocinetics:
Pages 31 ff., Section 2.6.1 Summary of ADME in Mammals:
Unfortunately, the in vitro comparative biotransformation study was unable to mimic the conversion of Diaminozide into UDMH and NDMA as observerd in vivo. As this in vitro study was performed with hepatocytes, the possibilities of extrahepatic conversion should be con-sidered to explain the findings in vivo. This may include metabolism in the intestine as well as the lung (which appears to be a potential target organ of diaminozide carcinogenicity). It appears unlikely that the slow hydrolysis observed in cell free incubations in vitro can explain the substantial conversion rate in the intact animal. Information on stability/hydrolysis at different pH may be useful in this context. Finally, it may be investigated whether there is any suitable biomonitoring or other human data that may useful to clarify whether for-mation of UDMH and NDMA also occurs in man.

Dossier Submitter's Response

Response from the applicant:
In the study of Riggs (2010; study no. GRL-12838), the melting point range of daminozide was determined to be 153-154.5 degrees C. The apparent inconsistency between this result and the result obtained in the earlier boiling point study of Riggs (2003; study no. GRL-12029) is discussed in the melting point study report.

To assess the reason why a higher value for melting point than boling point was determined, in the melting point study, a sample of test item was heated in an oven set at 156 degrees C until it melted. Aliquots of the test item and this melted test item were dissolved in dimethyl sulfoxide-D6 + 0.05% v/v TMS and NMR spectra of both solutions were taken.

The NMR spectra obtained were distinctly different indicating that the melting of the test item was obviously accompanied by immediate decomposition. The conclusion of the boling point study was that decomposition of daminozide had occurred in the temperature range of 142-145 degrees C. However, the melting point study showed that daminozide decomposes immediately upon melting. The conclusion can be made therefore that in the boiling point study, prior to the observed boiling range, decomposition had already occurred.

For the endpoints relative density, viscosity and granulometry it is stated that they are not a requirement according to 283/2014. This is a typo as the correct regulation is 283/2013 - will be corrected.

Toxicocinetics:
Based on the results of *in vitro* comparative metabolism study neither the possibility of extrahepatic conversion of daminozide to UDMH nor metabolism of daminozide by liver enzymes can be excluded. It is true that the presence of metabolites in the control samples without hepatocytes (not only in the treated ones) could indicate that the parent compound is rather degraded than metabolised by hepatocyte enzymes. However, the incubation time of hepatocytes with daminozide was only 3 hours. As indicated in the study by *Connor (2012)* the hydrolysis in aqueous solution from parent molecule to UDMH is characterized by maximum hydrolytic conversion between 4–24 hours (0.1% of parent molecule at 24h). In the toxicokinetic study *(Slauter, 1993)* high UDMH urinary excretion in time intervals 6–12h (8.14% of administered dose of daminozide) and 12–24h (17.4% of administered dose of daminozide) was observed, which suggests that UDMH may be *in vivo* product of metabolism since it is rather unlikely that hydrolysis *in vivo* would be much faster than spontaneous hydrolysis in aqueous solution. It is true that using

hepatocytes, 2-4 hour incubation is generally recommended. However, *Whalley et al. (2017)* claim that longer incubation times may be used dependent on the model system (e.g. plated hepatocytes) or testing laboratory protocol. At the EFSA workshop (on *in vitro* comparative metabolism) it was also said that there are two possibilities why no metabolism was observed: (i) the chemical is not metabolised at all; (ii) metabolism is not observed because of technical limitations of the method (e.g. limited incubation time, cells not viable, enzymes not expressed, etc.).
As UDMH was found *in vivo* in both rats and minipigs, it is highly likely that formation of UDMH also occurs in humans.

RAC's response

Thank you for your comment. RAC notes the clarification provided by the applicant concerning the apparent inconsistency between the values for melting point and for decomposition temperature. As for the comparative toxicokinetic study *in vitro*, RAC supports the view of DS that metabolism was not observed possibly because of some technical shortcomings of the test method (e.g. limited incubation time, cells not viable, enzymes not expressed, etc.). Unfortunately, there are no studies to support the hypothesis of extrahepatic metabolism. RAC agrees that metabolic formation of UDMH is likely to occur also in humans, and notes that no biomonitoring data are available for this evaluation.

Date	Country	Organisation	Type of Organisation	Comment number
23.09.2019	Norway		MemberState	3

Comment received

Volume 1 Level 2, 2.1.1. Metabolism:
Norway agrees with the RMS conclusion that no information on the role of UMDH in human metabolism can be extracted from the in vitro comparative study. Notably, some of the genotoxicity studies have been carried out with exposure durations that are not optimal with respect to UDMH (see comments on genotoxicity below) and therefore no firm conclusion regarding UDMH can be drawn based on those studies as well. This should be pointed out in the RMS concluding remarks for those studies.

Dossier Submitter's Response

Thank you for the supportive comment.
Separate *in vitro* genotoxicity studies are available for daminozide and UDMH. Therefore, the genotoxic potential of UDMH was sufficiently investigated. (Note: We do not agree with the applicant that the endpoint UDMH genotoxicity can be concluded). It is true that during the short-term treatment of *in vitro* mammalian chromosome aberration test the incubation with daminozide lasted only 2 hours instead of 3-6 hours (deviation from the OECD 473), whereas the hydrolysis in aqueous solution from daminozide to UDMH is characterized by maximum hydrolytic conversion between 4–24 hours. Thus, theoretically evaluation of UDMH impact on daminozide genotoxicity in this part of the test might be compromised due to shorter incubation time and consequent lower amount of UDMH formed by hydrolysis. However, during the long-term treatment of this test the cells were exposed to daminozide for 8 hours. (Note: 8-hour exposure to daminozide during long-term treatment also represents deviation from OECD TG 473 since the cells should be continuously exposed to the test substance until sampling at time equivalent to about 1.5 normal cell cycle lengths, which is approximately 18 hours for CHO cells).

RAC's response

Thank you for your comment. Considering the low metabolic and slow hydrolytic conversion rates of daminozide, RAC agrees that duration of some genotoxicity tests

might have been too short with respect to potential UDMH formation. Nevertheless, as also pointed out by the DS, there are sufficient additional studies investigating the genotoxic potential of UDMH, and the majority of them were negative *in vitro*.

CARCINOGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
24.09.2019	Germany		MemberState	4

Comment received

Page 87, Section 2.6.5.2 Comparison with the CLP criteria regarding carcinogenicity:
There is limited correlation between daminozide exposure and increased incidence of alveo-lar bronchiolar adenoma/carcinoma in female mice (single species, sex, and site – also see comments below). Accordingly, the available cancer bioassay data for daminozide alone would not support classification as Carc. 1B and Carc. 2 may be more appropriate. However, there may be "sufficient" strength of evidence justifying classification as Carc. 1B if it can be demonstrated that the formation of UDMH as reported for rats and minipigs and its further conversion into NDMA as reported minipigs can reasonably be expected to occur in humans as well. Both substances are presumed to be carcinogenic in humans (Carc. 1B). Unfortu-nately, the in vitro comparative biotransformation study using hepatocytes was not suitable to mimic the in vivo situation. This also casts doubt on the sensitivity of the mutagenicity assays using liver S9 mix in this particular case and a conclusion on genotoxic vs. non-genotoxic mode of action may not be possible when bearing this in mind.
Pages 77-81, Section 2.6.5.1 (2-year carcinogenicity study in rats):
It cannot be conclusively determined from this study that the increased incidence of pituitary adenomas in female rats was related to daminozide exposure. There was no clear monoton-ic dose-response relationship observed for pituitary adenomas in female rats. Even though there were significant increases in pituitary adenoma incidence in the middle 3 dose groups (i.e. 100, 500, 5000 ppm), only a subset of these animals was examined for microscopic find-ings. It is unclear as to how these animals were selected for histopathology and raises doubt in the interpretation of this finding. Furthermore, there was no significant increase in pitui-tary adenomas between control and the highest dose (10000 ppm) females and the inci-dence of 46.6 % was only slightly higher than the 44 % in control female F344 rats from Haseman, 1984 (mentioned in page 77).
Pages 81-86, Section 2.6.5.1 (2-year carcinogenicity study in mice):
We agree that the data indicate a neoplastic potential in mice. However, there are some limitations:
The incidence of alveolar bronchiolar adenoma/carcinoma in control male mice (50 %) ex-ceeded the historical control data (average of 33.4 %; max of 43.8 %) as reported in Maita, 1984 (page 86).
Numerical values for alveolar bronchiolar adenoma and adenoma and carcinoma in male mice did not show strict dose dependency and a statistically significant increase over the concurrent control was observed at 6000 but not at 10000 ppm for males.
When only lung carcinoma are considered, there was not statistically significant increase over concurrent controls. However, there may have been a trend to higher incidences. A statistical dose-response analysis for all lung tumors may assist in the final evaluation of this study.
Other: Pages 77, Section 2.6.5.1 Short summary and overall relevance of the provided in-formation on long-term toxicity and carcinogenicity – It is not clear on the rationale for se-lecting 100 ppm (5 mg/kg bw) as the provisional NOAEL. Aside from pituitary adenomas, there were also bile duct hyperplasia and ovarian effects (atrophy and cysts) observed in female rats exposed to daminozide. From the data presented in Table 2.6.5.1-3, it appears that these effects were already observed at the lowest tested

concentration of 100 ppm (e.g. 5/50 in controls vs. 16/49 in 100 ppm-exposed females for mild bile duct hyperplasia). This would mean that 5 mg/kg bw would be more suitable as a LOAEL rather than a provisional NOAEL.

Dossier Submitter's Response

As UDMH was found *in vivo* in both rats and minipigs, it is highly likely that the formation of UDMH also occurs in humans (however, there is not a proof). Nevertheless, in our opinion, classification for carcinogenicity is primarily based on the results of carcinogenicity studies.

Genotoxicity tests: It is true that during the short-term treatment of *in vitro* mammalian chromosome aberration test (in the presence of S-9 mix)the incubation with daminozide lasted only 2 hours instead of 3-6 hours (deviation from the OECD 473), whereas the hydrolysis in aqueous solution from daminozide to UDMH is characterized by maximum hydrolytic conversion between 4–24 hours. Thus, theoretically evaluation of UDMH impact on daminozide genotoxicity in this part of the test might be compromised due to shorter incubation time and consequent lower amount of UDMH formed by hydrolysis. In addition, we agree that it is doubtful whether S-9 mix fulfilled its purpose taking into account the results of *in vitro* comparative metabolism study, i.e. metabolism by liver enzymes not shown after 3-hour incubation. It should be noted that during the long-term treatment of this test (in the absence of S-9 mix) the cells were exposed to daminozide for 8 hours. (Note: 8-hour exposure to daminozide during long-term treatment also represents deviation from OECD TG 473 since the cells should be continuously exposed to the test substance until sampling at time equivalent to about 1.5 normal cell cycle lengths, which is approximately 18 hours for CHO cells).

2-year carcinogenicity study in rats: Based on the explanation of the applicant *(please, see comment number 8)* we understand that at mid-doses (100, 500, and 5000 ppm) only females with macroscopic abnormalities in pituitary + females that died during the study were subjected to histological examination of pituitary. In this case, it is clear that incidence of pituitary adenomas at these doses expressed in percentages is misleading, basically useless, and inappropriate for the setting of NOAEL for carcinogenicity. The incidence of pituitary adenomas at the top dose was non-significantly increased comparing to the concurrent control (37.3% vs. 46.6%) and slightly higher than spontaneous incidence retrieved by the RMS from the literature (36%: *Sandusky, 1988*; 42%: textbook of toxicology, *Hayes, 2014*; 44%: *Haseman, 1984*). The applicant was kindly asked during Public consultation to submit relevant HCD, i.e. from the respective laboratory. (HCD were not provided on the request during "Consultation period with the applicant"). HCD could be helpful in making decision whether these effects can be discounted or not. It should be noted that increased incidence of pituitary adenoma was also observed in the rat carcinogenicity study with daminozide metabolite UDMH (statistically significantly in female top dose group compared to controls; p=0.007).

2-year carcinogenicity study in mice: We remain of the opinion that increased incidence of pulmonary tumours (adenomas and adenomas combined with carcinomas) in each treated group in both sexes is treatment-related. The incidence of adenomas in the male concurrent control (40%) is within the range of HCD provided in the study report (18.2–44% in males). It is true that the incidence of alveolar bronchiolar adenomas combined with carcinomas in male controls exceeds range of provided HCD (19.1–44%). However, the upper level of this HCD range is not higher comparing to that for adenomas alone. Nevertheless, the incidence of adenomas combined with carcinomas in treated groups is higher than concurrent control as well as HCD range.

We agree that the lowest dose tested in the rat study, i.e. 100 ppm equal to 5 mg/kg bw/day is the LOAEL for systemic toxicity based on the increased incidence of bile duct hyperplasia in females. The NOAEL for carcinogenicity will be changed based on the explanation of the applicant why lower number of animals was examined at mid-doses. The applicant was kindly asked to submit relevant HCD for pituitary adenomas *(see above)*.

We admit that classification in category 2 could be more appropriate than in category 1B.

RAC's response

Thank you for your detailed analysis. RAC agrees that, while the formation of potentially carcinogenic metabolites is part of an overall weight of evidence assessment, conclusion on classification for carcinogenicity should be primarily based on the results from testing with the investigated substance. With respect to the increased incidences of pituitary adenomas in female Fischer rats, RAC considers the explanation provided by the applicant plausible (see comment number 8), and does not include this finding as evidence supporting classification for carcinogenicity. RAC also notes that the increased incidence rates for alveolar/bronchiolar adenoma and adenoma/carcinoma combined in male mice were statistically significant only at 6000 but not at 10000 ppm, and did not show dose dependency. While this study on its own does not provide a strong definitive support for carcinogenicity classification, the data indicate a neoplastic potential in mice and therefore was considered by RAC in the overall assessment of daminozide.

Date	Country	Organisation	Type of Organisation	Comment number
24.09.2019	France		MemberState	5

Comment received

FR: 2-year rat study:
Relevant laboratory historical control data should be submitted in order to assess the treatment relationship of the increased incidence of pituitary adenomas observed in all treated groups in a non-dose-related manner.

2-year mouse study:
FR agrees that the increased incidence of alveolar/bronchiolar adenomas/carcinomas (above laboratory HCD) observed in all treated groups should be considered treatment-related. The apparent lack of dose-relationship could be the consequence of the higher mortality observed in the 2 highest dose groups. Furthermore, this type of tumor being also observed with the major metabolite UDMH, a relationship to treatment with daminozide cannot be excluded.
Moreover, other neoplasms showed increased incidences in the treated groups and it would be interesting to compare their incidences with relevant laboratory historical control data, e.g. hemangiosarcomas in the uterus and liver (also observed in the study with UDMH).

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 rats whereas adenocarcinomas of the uterine endometrium and leiomyosarcomas of the uterus were observed in female mice.

Dossier Submitter's Response

2-year carcinogenicity study in rats: Based on the explanation of the applicant *(please, see comment number 8)* we understand that at mid-doses (100, 500, and 5000 ppm) only females with macroscopic abnormalities in pituitary + females that died during the

study were subjected to histological examination of pituitary. In this case, it is clear that incidence of pituitary adenomas at these doses expressed in percentages is misleading, basically useless, and inappropriate for the setting of NOAEL for carcinogenicity. The incidence of pituitary adenomas at the top dose was non-significantly increased comparing to the concurrent control (37.3% vs. 46.6%) and slightly higher than spontaneous incidence retrieved by the RMS from the literature (36%: *Sandusky, 1988*; 42%: textbook of toxicology, *Hayes, 2014*; 44%: *Haseman, 1984*). The applicant was kindly asked during Public consultation to submit relevant HCD, i.e. from the respective laboratory. (HCD were not provided on the request during "Consultation period with the applicant"). HCD could be helpful in making decision whether these effects can be discounted or not. It should be noted that increased incidence of pituitary adenoma was also observed in the rat carcinogenicity study with daminozide metabolite UDMH (statistically significantly in female top dose group compared to controls; p=0.007).

2-year carcinogenicity study in mice: Thank you for the supportive comment. It is true that non-significant increase in the incidence of liver haemangiosarcoma (in both sexes) and uterus haemangiosarcoma was observed in the top dose group comparing to the control. HCD are not available.

NTP studies: The results of mentioned studies were discounted by PPR Panel (2004): "There was a slight increase in uterine tumours in rats that was not statistically significant, and the increase in liver tumours in male mice was not considered significant because of the spontaneous rate and variability of this tumour type".

RAC's response

Thank you for your comments. With respect to the increased incidences of pituitary adenomas in female Fischer rats, RAC considers the additional information provided by the applicant plausible (see comment number 8), and does not include this tumour type as evidence supporting classification for carcinogenicity. RAC agrees that a relationship between daminozide treatment and the incidence of alveolar/bronchiolar adenoma and carcinoma in mice cannot be excluded. Therefore, this information is included in the weight of evidence assessment. A positive trend in the life table tests for liver haemangiosarcoma alone and for combined haemangiomas/haemangiosarcomas was observed in male mice, however the effect was not considered biologically significant due to the lack of statistical significance in the pairwise test. The increased incidences of adenocarcinomas of the uterine endometrium and leiomyosarcomas of the uterus in rats reported in the earlier NTP studies were considered as evidence supporting classification for carcinogenicity.

Date	Country	Organisation	Type of Organisation	Comment number
27.08.2019	Finland		MemberState	6

Comment received

There are two animal studies available investigating the carcinogenic potential of the substance daminozide. In a combined chronic toxicity and carcinogenicity study (in accordance with OECD TG 453), an increased incidence of pituitary adenomas (statistically significant at low and middle doses) was observed in Fischer 344 female rats. Although the strain is considered to be somewhat susceptible for this tumour type, the incidence was still higher when compared to concurrent controls and spontaneous tumour incidence in the literature. In a standard carcinogenicity study (in accordance with OECD TG 451), alveolar/bronchiolar adenomas and carcinomas were observed in both sexes in each treated group in CD1 mice. The increased incidence was statistically significant at

middle dose in males and at middle and high doses in females.

No data are available on the carcinogenicity of the substance in humans. Data on genotoxicity of daminozide is negative, and the underlying mechanism for carcinogenicity is not known. The major metabolite of daminozide, N,N-dimethylhydrazine (UDMH), is classified as Category 1B Carcinogen in the Annex VI under the CLP Regulation. The evidence from two animal species demonstrate that exposure to daminozide may lead to increased incidence of neoplasms, and the relevance to humans cannot be excluded. Category 1B for carcinogenicity is for substances presumed to have carcinogenic potential for humans, with classification largely based on animal evidence. The mode of non-genotoxic carcinogenic action of daminozide cannot be elucidated neither for pituitary adenomas nor alveolar/bronchiolar adenomas and carcinomas. FI CA supports the proposed classification of Carc. 1B for daminozide.

Dossier Submitter's Response

Thank you for the supportive comment. Nevertheless, we have changed our opinion. We think that classification in category 2 could be more appropriate (please, see other comments on carcinogenicity).

RAC's response

Thank you for your clear position on classification for carcinogenicity. After review of the supplemental information provided during PC, RAC considers that classification as Carc. 2 is more appropriate. For details on the studies addressed by you, please see RAC's response to the earlier comments.

Date	Country	Organisation	Type of Organisation	Comment number
23.09.2019	Norway		MemberState	7

Comment received

2.6.5.1 Long-term toxicity and carcinogenicity:
2-year rats: We are uncertain as to whether daminozide should be considered as carcinogenic based on the findings presented in this study. What are the reasons for not examining all the mid-dosed animals for pituitary adenomas? In addition, the prevalence of these adenomas is quite high also in the control animals. Are there any valid historical control data available with respect to this effect?

2-year mice: We are uncertain as to whether daminozide should be considered as carcinogenic based on the findings presented in this study, considering that it has been reported that these kind of tumors occur spontaneously in many strains of mice, and the incidence varies between strain with a higher incidence in the males compared to the females. The daminozide CLH-dossier contains historical control data for this effect and indicates a large variability between studies. In addition, in particular for the female rats, the HCD does not fit with the control, and does not support the suggested effect of the study.

Norway think that the evidence for carcinogenic effects of daminozide from the chronic studies in rats and mice is doubtful and requires more discussion. 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.

Dossier Submitter's Response
2-year carcinogenicity study in rats: The increased incidence of pituitary adenomas at mid-doses was re-evaluated based on the explanation of the applicant why lower number of animals was examined at these doses *(please see the comment number 8 for details*). The incidence of pituitary adenomas at the top dose was non-significantly increased comparing to the concurrent control (37.3% vs. 46.6%) and slightly higher than spontaneous incidence retrieved by the RMS from the literature (36%: *Sandusky, 1988*; 42%: textbook of toxicology, *Hayes, 2014*; 44%: *Haseman, 1984*). The applicant was kindly asked during "Public consultation" to submit relevant HCD, i.e. from the respective laboratory (HCD were not provided on the request during "Consultation period with the applicant").HCD could be helpful in making decision whether these effects can be discounted or not. Only HCD retrieved from the literature by the RMS are available. It should be noted that increased incidence of pituitary adenomas was also observed in the rat carcinogenicity study with daminozide metabolite UDMH (statistically significant in female top dose group compared to controls; p=0.007). 2-year carcinogenicity study in mice: We remain of the opinion that the increased incidence of pulmonary tumours in mice is treatment related. Although alveolar/bronchiolar adenoma belongs to common neoplasms in CD-1 male mice, CD-1 mice are considered to represent less susceptible strain *(for details see comment number 8).*The alveolar/bronchiolar adenoma incidence is increased above the concurrent as well as HCD in each treated group in both sexes (i.e. also in females despite the fact that the incidence in controls was too high). This effect is also evident (to a greater extent) after combination of adenomas with carcinomas (statistical significant at two highest doses). Pulmonary neoplasms were also found in studies with UDMH (Carc. 1B). In general, it is not uncommon that one sex is more prone to tumour occurrence. As UDMH was found *in vivo* in both rats and minipigs, it is highly likely that the formation of UDMH also occurs in humans (however, there is not a proof). Thus, the contribution of daminozide metabolite UDMH to the carcinogenic potential cannot be excluded. In our opinion, the active substance in general should be classified even if its metabolite arising in the body after the active substance exposure is responsible for the carcinogenic effect. We admit that classification in category 2 could be more appropriate.
RAC's response
RAC considers the additional information provided by the applicant plausible (see comment number 8), and does not include the reported incidences of pituitary adenomas in Fischer rats as evidence supporting classification for carcinogenicity. RAC agrees with DS that a possible relationship between daminozide treatment and the occurrence of alveolar/bronchiolar adenoma and carcinoma in mice cannot be excluded, therefore these findings are considered in a weight of evidence assessment. The potential contribution of the carcinogenic metabolite UDMH to the toxicity profile of daminozide is also addressed in the opinion document.

Date	Country	Organisation	Type of Organisation	Comment number
16.09.2019	United Kingdom	Daminozide Task Force	Company-Manufacturer	8

Comment received

Applicant:
Disagrees with the proposal for Carc 1B. There is no evidence of carcinogenicity in either rats or mice. In the absence of evidence, "No classification" is appropriate.

There is no neoplastic effect in the rat study; the data are mis-interpreted.
By design, the study guideline permits that tissues of the terminal sacrifice animals (largely still healthy at the end of the study) be histologically examined only in the control and high dose animals; intermediate groups are examined only for target tissues and macroscopic abnormalities; any animal dying during the study (in any group) is subject to complete histological examination of all tissues. Pituitary tumours are easily detected at necropsy (a pituitary swells to 5 or 10 times normal size and very evidently overgrows its bony fossa at the base of the brain); all tissues are harvested and retained (therefore: examined), even if not progressing to histology. All pituitary tumours are therefore found, in all rats. As abnormalities, they all progress to histopathology. The incidence of pituitary tumours therefore is approximately equivalent (and entirely normal) across all groups; there is no disturbance of the pituitary tumour profile.
The type of tumour analysis offered in the RAR – tumours limited to intermediate doses – is incompatible with the study guideline. By guideline, the histological examination at intermediate doses is not comprehensive. It is fundamentally incorrect to attempt a tumour analysis on a selectively incomplete data set, which by design is skewed to tissues showing abnormalities.

There is no neoplastic effect in the mouse. The pulmonary tumours show an incidental distribution of a common tumour in this strain, without dose relationship despite substantial dose spacing. The lung tumour burden in this study was unusually high compared to HCD even in controls. With specific respect to the criteria offered in the EFSA "Guidance on the Application of CLP Criteria", the pulmonary tumours in this mouse study have an exceptionally high spontaneous rate. Applicant notes the top dose in mice was approximately 1500 mg/kg bw/day, and recommends that even the most severe interpretation of the tumour distribution meets the situation for "no classification" where: "appearance of only spontaneous tumours, especially if they appear only at high dose levels, may be sufficient to downgrade a classification from Category 1B to Category 2, or even no classification" (p.382 of July 2017 Guidance).

However, Applicant supports and recommends the more comprehensive evaluation of these identical data in the "Opinion of the Scientific Panel on Plant Health, Plant Protection Products and their Residues on a request from the Commission related to the evaluation of daminozide in the context of Council Directive 91/414/EEC, EFSA Journal (2004), 61, 1-27."
"In the more recent studies conducted according to Good Laboratory Practice (GLP) standards
(Johnson, 1988), where the purity of the test material (daminozide) was known, no statistically
significant increase in tumour incidences was reported. These two studies used daminozide
technical material of known purity, containing typical amounts of the impurity UDMH (~30 ppm).
No significant oncogenic effects were found in either Fischer 344 rats or CD-1 mice fed up

to
10,000 ppm (corresponding respectively to about 500 and 1,500 mg daminozide/kg bw per
day)."

"The PPR Panel concluded that these studies do not provide any evidence that daminozide induces carcinogenic effects in rats and mice."

Under CLP, "no evidence" of carcinogenicity results in "No classification" for carcinogenicity.

ECHA note – An attachment was submitted with the comment above. Refer to public attachment PPR opinion - daminozide 2004.pdf

Dossier Submitter's Response

Based on the explanation of the applicant we understand that at mid-doses (100, 500, and 5000 ppm) only females with macroscopic abnormalities in pituitary + females that died during the study were subjected to histological examination of pituitary. In this case, it is clear that incidence of pituitary adenomas at these doses expressed in percentages is misleading, basically useless, and inappropriate for the setting of NOAEL for carcinogenicity. The incidence of pituitary adenomas at the top dose was non-significantly increased comparing to the concurrent control (37.3% vs. 46.6%) and slightly higher than spontaneous incidence retrieved by the RMS from the literature (36%: *Sandusky, 1988*; 42%: textbook of toxicology, *Hayes, 2014*; 44%: *Haseman, 1984*). The applicant was kindly asked to submit relevant HCD (from the respective laboratory). HCD could be helpful in making decision whether these effects can be discounted or not. It should be noted that increased incidence of pituitary adenoma was also observed in the rat carcinogenicity study with daminozide metabolite UDMH (statistically significantly in female top dose group compared to controls; p=0.007).

We do not agree that no neoplastic effect was observed in mice. 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 treatment-related. The incidence of adenomas in the male concurrent control is within the range of HCD provided in the study report (18.2–44% in males). 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 HCD (8.7 – 22%), does not correlate with the literature data (2–27%; *Giknis, 2005, Hayes, 2014*) and may skew the results (to lower the difference between treated group and controls). We admit that it could be more appropriate to classify daminozide in category 2, but not for reasons written by the applicant. (1) Although it is true that alveolar/bronchiolar adenoma belongs to the common neoplasms in CD1 male mice, CD1 mice are considered to represent less susceptible strain. 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)*. (2) The increased incidence of alveolar/bronchiolar adenomas as well as alveolar/bronchiolar adenomas combined with carcinomas was increased already at the lowest dose of 45 mg/kg bw/day (i.e. tumours did not occur only at the top dose). In addition, this type of tumour was also found in studies with UDMH (Carc. 1B).

Although the PPR Panel concluded that daminozide is not carcinogenic, they noted that: „The results provided by direct long-term testing of UDMH in rodents are in apparent

discrepancy with those obtained by testing daminozide. In fact, the daminozide long-term studies were negative in rats and mice at doses that should have produced internal doses of metabolically-formed UDMH at least one order of magnitude higher than those proven to be carcinogenic on direct testing". Furthermore, it should be noted that the statement of PPR Panel on the statistical significance ("No statistically significant increase in tumour incidences was reported" in studies with daminozide) is inaccurate since Fisher exact test revealed the statistically significant increase in incidence of alveolar/bronchiolar 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).
RAC's response
Thank for providing further details on the study in rats. RAC considers that this additional information plausibly explains the terminal incidence rates of 100% for pituitary adenomas in females, and does not include this tumour type as evidence supporting classification for carcinogenicity. RAC does not agree that the pulmonary tumours in mice show solely an incidental distribution of a common tumour type in this strain. Therefore, the increased incidence of alveolar/bronchiolar adenoma and carcinoma is included in a weight of evidence assessment. Additional aspects of the study such as the high background and concurrent control incidences, unclear dose response, and the specific susceptibility of the strain to lung tumours are considered during this evaluation. Reference to previous assessments of daminozide is included in the opinion document.

Date	Country	Organisation	Type of Organisation	Comment number
23.09.2019	Denmark		MemberState	9
Comment received				
DK proposal: Carc. 2 - H351 Neoplasia is observed in both rats (pituitary adenomas) and mice (adenoma and carcinoma in lungs) in the studies CLH report but in neither species is a clear dose-response relationship present. Based on the results, a carcinogenic potential of daminozide cannot be ruled out but DK do not believe there is sufficient evidence for a classification as Carc. 1B as proposed by the dossier submitter. DK proposes that Daminozide should be classified as Carc. 2.				
Dossier Submitter's Response				
We agree that the classification in Category 2 could be more appropriate (please, see also other comments on carcinogenicity).				
RAC's response				
Thank you for a clear position on the classification proposal. RAC shares the view that a carcinogenic potential for daminozide cannot be ruled out. After review of the supplemental information provided during PC, RAC considers that classification as Carc. 2 is more appropriate.				

MUTAGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
23.09.2019	Denmark		MemberState	10
Comment received				
No classification necessary Both in vitro and in vivo studies presented in the CLH report were negative.				
Dossier Submitter's Response				
Noted				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
16.09.2019	United Kingdom	Daminozide Task Force	Company-Manufacturer	11
Comment received				
Applicant: Agrees "No classification" for genotoxicity/ germ cell mutation Vol.1 Level 2 2.6.4.3: Germ cell mutagenicity ECHA note – An attachment was submitted with the comment above. Refer to public attachment PPR opinion - daminozide 2004.pdf				
Dossier Submitter's Response				
Noted				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
24.09.2019	Germany		MemberState	12
Comment received				
Pages 67 ff., Section 2.6.4.1 Short summary and overall relevance of the provided information on genotoxicity: The available data do not warrant classification for mutagenicity. At the same time, it should be taken into consideration that the in vitro comparative biotransformation study using hepatocytes did not lead to generation of quantifiable levels of UDMH and NDMA unlike in vivo studies in minipigs and rats. Thus, the in vitro mutagenicity studies using liver S9 mix may, likewise, not have been suitable to predict in vivo genotoxicity potential of daminozide. For the only acceptable in vivo study that was performed by i.p. injection in mice (Anony-mous 2003), it is not clear whether UDMH and NDMA have been formed.				
Dossier Submitter's Response				
It is true that during the short-term treatment of *in vitro* mammalian chromosome aberration test (in the presence of S-9 mix)the incubation with daminozide lasted only 2 hours instead of 3-6 hours (deviation from the OECD 473), whereas the hydrolysis in aqueous solution from daminozide to UDMH is characterized by maximum hydrolytic conversion between 4–24 hours. Thus, theoretically evaluation of UDMH impact on daminozide genotoxicity in this part of the test might be compromised due to shorter incubation time and consequent lower amount of UDMH formed by hydrolysis. In addition, we agree that it is doubtful whether S-9 mix fulfilled its purpose taking into account the results of *in vitro* comparative metabolism study, i.e. metabolism by liver enzymes not shown after 3-hour incubation. It should be noted that during the long-term treatment of				

this test (in the absence of S-9 mix) the cells were exposed to daminozide for 8 hours. (Note: 8-hour exposure to daminozide during long-term treatment also represents deviation from OECD TG 473 since the cells should be continuously exposed to the test substance until sampling at time equivalent to about 1.5 normal cell cycle lengths which is approximately 18 hours for CHO cells).
The only acceptable *in vivo* genotoxicity study with daminozide (combined micronucleus and chromosome aberration test) used intraperitoneal route of administration, thus first-pass effect was not involved.

RAC's response

RAC agrees that the contribution of metabolically formed UDMH to daminozide genotoxicity *in vitro* might have been rather limited due to the reasons explained by the DS. Nevertheless, *in vitro* genotoxicity studies with UDMH were provided in the dossier, and they did not demonstrate a clear positive response. RAC notes that the available *in vivo* combined micronucleus and chromosome aberration test was negative as well, which lowers to some degree the concerns associated with the limitations of the *in vitro* tests.

Date	Country	Organisation	Type of Organisation	Comment number
23.09.2019	Norway		MemberState	13

Comment received

Volume 1 Level 2, 2.6.4.1 genotoxicity/germ cell mutagenicity:
The bacterial reverse mutation assays were conducted with 2-aminoanthracene as the only indicator of S9 mix efficacy. According to the guidelines, each batch of S9 should in that case also be characterized with a mutagen that requires metabolic activation by microsomal enzymes. This has not been provided, and the possible impact of this limitation on the interpretation of the study results has not been discussed.

With regard to UDMH and the discussion on incubation time of daminozide in cell culture (see RMS comments on the In vitro comparative metabolism study in section B.6.1.1 in the RAR), it should be noted that the genotoxicity studies conducted according to OECD 476 (exposure 4 h ±S9) and OECD 473 (exposure 2 h, +S9), have exposure durations that are not optimal with respect to UDMH.

One study (The Ames metabolic activation test to assess the potential mutagenic effect of daminozide) was considered to be supplementary as "only 4 bacteria strains were used and the strain for detection of oxidizing and cross-linking agents was not involved."
Another study (The Salmonella/mammalian-microsome plate incorporation mutagenicity assay (Ames test)) was considered acceptable, but has the same deviation. Since none of the studies included a strain with AT base pair they should both be considered supplementary. In our opinion, a bacterial reverse mutation test using four strains of S. typhimurium together with TA 102 or E.coli WP2 should have been conducted with suitable positive control reference substance(s) for S9 (e.g. not 2-aminoanthracene as the sole indicator of the efficacy of the S9-mix) .

Dossier Submitter's Response

The only positive control in Ames tests for samples in the presence of S-9 mix was 2-aminoanthracene. However, according to the study reports: "The S-9 homogenate was characterized for its ability to metabolize the pro-mutagens
7, 12-dimethylbenzanthracene and 2-aminoanthracene to mutagens" (prior to use in Ames tests).

Separate *in vitro* genotoxicity studies are available for daminozide and UDMH. Therefore, relating solely to the comment of NO, the genotoxic potential of UDMH was sufficiently investigated. (Note: We do not agree with the applicant that the endpoint UDMH genotoxicity can be concluded). It is true that during the short-term treatment of *in vitro* mammalian chromosome aberration test (in the presence of S-9 mix) the incubation with daminozide lasted only 2 hours instead of 3-6 hours (deviation from the OECD 473), whereas the hydrolysis in aqueous solution from daminozide to UDMH is characterized by maximum hydrolytic conversion between 4–24 hours. Thus, theoretically evaluation of UDMH impact on the daminozide genotoxicity in this part of the test might be compromised due to shorter incubation time and consequent lower amount of UDMH formed by hydrolysis. In addition, it is doubtful whether S-9 mix fulfilled its purpose taking into account the results of *in vitro* comparative metabolism study, i.e. metabolism by liver enzymes not shown after 3-hour incubation. It should be noted that during the long-term treatment of this test (in the absence of S-9 mix) the cells were exposed to daminozide for 8 hours. (Note: 8-hour exposure to daminozide during long-term treatment also represents deviation from OECD TG 473 since the cells should be continuously exposed to the test substance until sampling at time equivalent to about 1.5 normal cell cycle lengths, which is approximately 18 hours for CHO cells). The study by *San (1991)* was regarded as acceptable although the strain for detection of crosslinking mutagens was not involved since the study with *Escherichia coli (Williams, 2006)* is available. The same should be applied for the study by *Richold (1984).* We apologise for this mistake (inconsistency). We do not agree that the new Ames test should be required.
RAC's response
Thank you for your analysis. RAC notes the response of DS regarding the usability of 2-aminoanthracene as a positive control and indicator of S9 mix efficacy, and is aware that exposure durations of the *in vitro* tests are possibly not optimal with respect to UDMH formation. RAC agrees with DS that apart from the discussed possibility of insufficient for metabolic conversion incubation time, genotoxicity of daminozide in the Ames tests is adequately covered.

TOXICITY TO REPRODUCTION

Date	Country	Organisation	Type of Organisation	Comment number
23.09.2019	Denmark		MemberState	14
Comment received				
No classification necessary 2-generation studies as well as prenatal development studies showed no effect on fertility, mating and development.				
Dossier Submitter's Response				
Thank you for the supportive comment.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
16.09.2019	United Kingdom	Daminozide Task Force	Company-Manufacturer	15
Comment received				
Applicant: Agrees "No classification" for reproductive toxicity Vol.1 Level 2 2.6.6.4: Reproductive toxicity ECHA note – An attachment was submitted with the comment above. Refer to public attachment PPR opinion - daminozide 2004.pdf				
Dossier Submitter's Response				
Thank you for the supportive comment.				
RAC's response				
Noted.				

RESPIRATORY SENSITISATION

Date	Country	Organisation	Type of Organisation	Comment number
23.09.2019	Denmark		MemberState	16
Comment received				
No classification necessary Based on acute inhalation toxicity, Buehler test and local lymph node assay, no potential of respiratory sensitization was observed.				
Dossier Submitter's Response				
Thank you for the supportive comment.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
16.09.2019	United Kingdom	Daminozide Task Force	Company-Manufacturer	17
Comment received				
Applicant: Agrees "No classification" for respiratory sensitisation Vol.1 Level 2 2.6.2.6.3: Respiratory sensitisation ECHA note – An attachment was submitted with the comment above. Refer to public attachment PPR opinion - daminozide 2004.pdf				
Dossier Submitter's Response				
Thank you for the supportive comment.				
RAC's response				
Noted.				

OTHER HAZARDS AND ENDPOINTS – Acute Toxicity

Date	Country	Organisation	Type of Organisation	Comment number
23.09.2019	Denmark		MemberState	18
Comment received				
No classification necessary				
Dossier Submitter's Response				
Thank you for the supportive comment.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
16.09.2019	United Kingdom	Daminozide Task Force	Company-Manufacturer	19
Comment received				
Applicant: Agrees "No classification" for acute oral toxicity Vol 1 Level 2 2.6.2.1.3: Acute oral toxicity Applicant: Agrees "No classification" for acute dermal toxicity Vol.1 Level 2 2.6.2.2.3: Acute dermal toxicity Applicant: Agrees "No classification" for acute inhalation toxicity Vol.1 Level 2.6.2.3.3: Acute inhalation toxicity Applicant: Agrees "No classification" for aspiration hazard although not opened for commenting as non-relevant Vol.1 Level 2 2.6.2.9.3: Aspiration hazard ECHA note – An attachment was submitted with the comment above. Refer to public attachment PPR opinion - daminozide 2004.pdf				
Dossier Submitter's Response				
Thank you for the supportive comment.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
23.09.2019	Norway		MemberState	20
Comment received				
Norway agrees with RMS that no classification is warranted.				
Dossier Submitter's Response				
Thank you for the supportive comment.				
RAC's response				
Noted.				

OTHER HAZARDS AND ENDPOINTS – Skin Hazard

Date	Country	Organisation	Type of Organisation	Comment number
23.09.2019	Denmark		MemberState	21
Comment received				
No classification necessary				
Dossier Submitter's Response				
Thank you for the supportive comment.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
16.09.2019	United Kingdom	Daminozide Task Force	Company-Manufacturer	22
Comment received				
Applicant: Agrees "No classification" for skin corrosion/ irritation Vol.1 Level 2 2.6.2.4.3: Serious eye damage/irritation ECHA note – An attachment was submitted with the comment above. Refer to public attachment PPR opinion - daminozide 2004.pdf				
Dossier Submitter's Response				
Thank you for the supportive comment.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
23.09.2019	Norway		MemberState	23
Comment received				
Norway agrees with RMS that no classification is warranted.				
Dossier Submitter's Response				
Thank you for the supportive comment.				
RAC's response				
Noted.				

OTHER HAZARDS AND ENDPOINTS – Eye Hazard

Date	Country	Organisation	Type of Organisation	Comment number
23.09.2019	Denmark		MemberState	24
Comment received				
No classification necessary Daminozide is a mild eye irritant but not enough to warrant classification				
Dossier Submitter's Response				
Thank you for the supportive comment.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
16.09.2019	United Kingdom	Daminozide Task Force	Company-Manufacturer	25
Comment received				
Applicant: Agrees "No classification" for serious eye damage/irritation Vol.1 Level 2 2.6.2.5.3: Serious eye damage/irritation ECHA note – An attachment was submitted with the comment above. Refer to public attachment PPR opinion - daminozide 2004.pdf				
Dossier Submitter's Response				
Thank you for the supportive comment.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
23.09.2019	Norway		MemberState	26
Comment received				
Norway agrees with RMS that no classification is warranted.				
Dossier Submitter's Response				
Thank you for the supportive comment.				
RAC's response				
Noted.				

OTHER HAZARDS AND ENDPOINTS – Skin Sensitisation Hazard

Date	Country	Organisation	Type of Organisation	Comment number
23.09.2019	Denmark		MemberState	27
Comment received				
No classification necessary Daminozide was negative in both a Buehler test and a local lymph node assay.				
Dossier Submitter's Response				
Thank you for the supportive comment.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
16.09.2019	United Kingdom	Daminozide Task Force	Company-Manufacturer	28
Comment received				
Applicant: Agrees "No classification" for skin sensitisation Vol.1 Level 2 2.6.2.7.3: Skin sensitisation ECHA note – An attachment was submitted with the comment above. Refer to public attachment PPR opinion - daminozide 2004.pdf				

Dossier Submitter's Response
Thank you for the supportive comment.
RAC's response
Noted.

Date	Country	Organisation	Type of Organisation	Comment number
23.09.2019	Norway		MemberState	29
Comment received				
Volume 1 Level 2, 2.6.2.7 Skin sensitization: It is concluded that daminozide is not a skin sinsitizer since it tested negative in both. We do not agree with this conclusion as we consider the concentrations tested in the LLNA as too low (only 5, 10 and 25 % has been tested). There was a dose –response. Are there any reasons for not testing higher concentrations in this case? The reasons for not testing higher concentrations should be included in the evaluation. There is not reported that daminozide causes skin irritation in other studies. Norway is of the opinion that the Buhler test reported is not as reliable for detecting skin sensitization. It has only three repeats in the induction phase, and there is only one challenge dose.				
Dossier Submitter's Response				
According to the original study report a preliminary screening test was performed. The mouse was treated by daily application of the test substance at the maximum suitable concentration (i.e. 25% m/v in DMSO) to the dorsal surface of each ear for 3 consecutive days. Death or signs of systemic toxicity/excessive irritation were not noted. Based on this information the dose levels selected for the main test were 5, 10, and 25% in DMSO. We understand that fewer inductions might lower the Buehler test sensitivity (non-guideline Buehler test variants with higher number of inductions are available). However, 3 inductions are in accordance with OECD TG 406. Therefore, this study is regarded as acceptable.				
RAC's response				
RAC notes that concentrations of only up to 25% have been used in the LLNA test, and the test results showed an apparent dose response. It is further noted that the study was performed according to the current guideline, and that the maximum suitable concentration for the study was established in a preliminary test. However, considering that the substance was not irritant up to 100% and the lack of plausible explanation for not using higher doses, RAC views the study as inadequate for classification purposes.				

OTHER HAZARDS AND ENDPOINTS – Specific Target Organ Toxicity Single Exposure

Date	Country	Organisation	Type of Organisation	Comment number
23.09.2019	Denmark		MemberState	30
Comment received				
No classification necessary				
Dossier Submitter's Response				
Thank you for the supportive comment.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
16.09.2019	United Kingdom	Daminozide Task Force	Company-Manufacturer	31
Comment received				
Applicant: Agrees "No classification" for STOT-SE Vol.1 Level 2 2.6.2.10.3: STOT-SE ECHA note – An attachment was submitted with the comment above. Refer to public attachment PPR opinion - daminozide 2004.pdf				
Dossier Submitter's Response				
Thank you for the supportive comment.				
RAC's response				
Noted.				

OTHER HAZARDS AND ENDPOINTS – Specific Target Organ Toxicity Repeated Exposure

Date	Country	Organisation	Type of Organisation	Comment number
23.09.2019	Denmark		MemberState	32
Comment received				
No classification necessary				
Dossier Submitter's Response				
Thank you for the supportive comment.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
16.09.2019	United Kingdom	Daminozide Task Force	Company-Manufacturer	33
Comment received				
Applicant: Agrees "No classification" for STOT-RE Vol.1, Level 2 2.6.3.1.3: STOT-RE Disagrees that the single renal cell adenoma in the one-year dog study is of any toxicological significance. Applicant agrees with the contrasting statement at in Daminozide _RAR_08 Vol_3CA B-6 p.30: "the renal cell adenoma in this female was considered to be a spontaneous tumour, which occurred by chance in the high dose group". As a spontaneous finding, it clearly does not support a toxicological conclusion. ECHA note – An attachment was submitted with the comment above. Refer to public attachment PPR opinion - daminozide 2004.pdf				
Dossier Submitter's Response				
Thank you for the supportive comment on STOT-RE. The mentioned "contrasting statement" was the conclusion of the study author. In our opinion, it cannot be excluded without any doubt that occurrence of uncommon tumour (renal cell adenoma) in one female of the highest dose group in the 1-year dog study is treatment-related.				
RAC's response				
RAC considers the single incidence of a renal cell adenoma in one female at the highest dose of 199 mg/kg bw/day in the 1-year dog study of rather low relevance for STOT RE classification.				

OTHER HAZARDS AND ENDPOINTS – Hazardous to the Aquatic Environment

Date	Country	Organisation	Type of Organisation	Comment number
23.09.2019	Denmark		MemberState	34
Comment received				
No classification necessary				
Dossier Submitter's Response				
Noted.				
RAC's response				
Thank you for your comment. The support for no classification of the substance as hazardous to the aquatic environment is noted by RAC. RAC agrees that daminozide does not warrant clasification for acute and chronic aquatic hazard.				

Date	Country	Organisation	Type of Organisation	Comment number
16.09.2019	United Kingdom	Daminozide Task Force	Company-Manufacturer	35
Comment received				
Applicant: Agrees "No classification" for environmental hazards within the scope of the CLH public consultation (ie opened for commenting) Hazardous to the aquatic environment Vol.1 Level 2 2.9.2.5 ECHA note – An attachment was submitted with the comment above. Refer to public attachment PPR opinion - daminozide 2004.pdf				
Dossier Submitter's Response				
Noted.				
RAC's response				
Thank you for your comment. The support for no classification of the substance as hazardous to the aquatic environment is noted by RAC. RAC agrees that daminozide does not warrant clasification for acute and chronic aquatic hazard.				

Date	Country	Organisation	Type of Organisation	Comment number
24.09.2019	United Kingdom		MemberState	36
Comment received				
Daminozide (ISO); 4-(2,2-dimethylhydrazino)-4-oxobutanoic acid; N-dimethylaminosuccinamic acid (EC: 216-485-9; CAS: 1596-84-5) The chronic fish toxicity study has been conducted with the formulation 'Dazide Enhance'. The relevance of this study for the classification of the active substance is unclear due to the presence of co-formulants. Please can the dossier submitter consider if the co-formulants in 'azide Enhance' influence the toxicity of daminozide?				
Dossier Submitter's Response				
All co-formulants present in formulation Dazide Enhance in amount >0.01% w/w do not require any classification. Therefore, their influence on the toxicity of daminozide is not considered relevant.				

RAC's response
Thank you for your comment. RAC notes that the comment refers to acute fish toxicity study on common carp (*Cyprinus carpio*) performed with formulation (Dazide Enhance). In the CLH report no chronic fish toxicity study with formulation Dazide Enhance is available. RAC considers that the information provided by the DS regarding the use of formulation Dazide Enhance in the key study as adequate to allow its use for classification.

Date	Country	Organisation	Type of Organisation	Comment number
24.09.2019	France		MemberState	37
Comment received				
FR: Based on the available toxicity data with the active ingredient, we agree with the classification proposal regarding environmental hazard. However, as daminozide is a plant growth regulator, toxicity data on aquatic macrophytes is required according to the regulation EU No 283/2013. Therefore, it would be useful to have this toxicity data in order to confirm the environmental classification of daminozide. An update would be necessary if a toxicity data on aquatic macrophyte is provided by the applicant during the on-going EU peer-review process linked to the renewal of daminazide according to Re EC No 1107/2009.				
Dossier Submitter's Response				
The comment is agreed. It is noted that the study has already been performed by applicant and it will be submitted and used in updated risk assessment and classification during the on-going peer-review process.				
RAC's response				
Thank you for your comment. The support for no classification of the substance as hazardous to the aquatic environment is noted by RAC. RAC agrees that daminozide does not warrant clasification for acute and chronic aquatic hazard. During the process of the preparation of the first draft opinion, RAC became aware of new experimental study Growth Inhibition of *Myriophyllum spicatum* in a Water/Sediment System (Schwarz, 2020) performed with formulation Alar 85 SG. RAC notes that toxicity values (see Table in ODD) from this study are above the trigger value of 1 mg/L for classification of the substance for acute and chronic hazard. Therefore, the results from this study do not affect the classification of daminozide proposed by DS.				

OTHER HAZARDS AND ENDPOINTS – Hazardous to the Ozone Layer

Date	Country	Organisation	Type of Organisation	Comment number
23.09.2019	Denmark		MemberState	38
Comment received				
No classification necessary The physical-chemical properties of daminozide do not suggest that the substance is volatile. Furthermore, half-life in air is short, so any volatilized material will be rapidly degraded.				
Dossier Submitter's Response				
Thank you for the supportive comment.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
16.09.2019	United Kingdom	Daminozide Task Force	Company-Manufacturer	39
Comment received				
Applicant: Agrees "No classification" for environmental hazards within the scope of the CLH public consultation (ie opened for commenting) Hazardous to the aquatic environment Vol.1 Level 2 2.8.3.1.2 ECHA note – An attachment was submitted with the comment above. Refer to public attachment PPR opinion - daminozide 2004.pdf				
Dossier Submitter's Response				
Noted.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
19.09.2019	Belgium		MemberState	40
Comment received				
Based on the available data given in the CLH dossier, BE CA supports the reasoning and conclusion on the classification of daminozide for the environment : classification is not warranted, both for aquatic acute and aquatic chronic toxicity. The CLH dossier contains a huge amount of information not relevant for classification purposes which hinder an efficient evaluation of the relevant data. Some editorial or/and minor comments : Table 61 : the LC50 value for Cyprinus carpio (2010) is expressed as mean measured concentration, while in the description it is mentioned that it is a nominal value.				
Dossier Submitter's Response				
Agreed, the typo will be corrected.				
RAC's response				
Noted.				

OTHER HAZARDS AND ENDPOINTS – Physical Hazards

Date	Country	Organisation	Type of Organisation	Comment number
23.09.2019	Denmark		MemberState	41
Comment received				
No classification necessary Daminozide showed no evidence of being explosive, fammable, self-reactive, pyrophoric, self-heating or oxidising, and the substance has no potential to emit flammable gases in contact with water since daminozide dissolves in water to form a stabile mixture.				
Dossier Submitter's Response				
Noted.				

RAC's response
Your comment is noted. However, RAC is unable to conclude on the hazard classes explosive and self-reactive substances due to the lack lack of/ insufficient data.

Date	Country	Organisation	Type of Organisation	Comment number
16.09.2019	United Kingdom	Daminozide Task Force	Company-Manufacturer	42
Comment received				
Applicant: Agrees "No classification" for any of the relevant physical hazard within the scope of the CLH public consultation (ie opened for commenting) Vol 1 Level 2 Explosive 2.2.1.1.1.1&2 Flammable solid 2.2.1.1.6.1&2 Self-reactive substances Pyrophoric solids 2.2.1.1.7 (Not relevant) Self-heating substance Substances which in contact with water emit flammable gases 2.2.1.1.10.1-2&3 (Not relevant) Oxidising solid 2.2.1.1.13.2&3 ECHA note – An attachment was submitted with the comment above. Refer to public attachment PPR opinion - daminozide 2004.pdf				
Dossier Submitter's Response				
Noted.				
RAC's response				
Noted. However, RAC is unable to conclude on the hazard classes explosive and self-reactive substances due to the lack lack of/ insufficient data.				

PUBLIC ATTACHMENTS
1. PPR opinion - daminozide 2004.pdf [Please refer to comment No. 1, 8, 11, 15, 17, 19, 22, 25, 28, 31, 33, 35, 39, 42]