Committee for Risk Assessment

RAC

Annex 3

Records

of the targeted public consultation following the submission of new information in relation to the carcinogenicity and mutagenicity hazard classes 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 3 RECORDS OF THE TARGETED PUBLIC CONSULTATION ON DAMINOZIDE (ISO); 4-(2,2-DIMETHYLHYDRAZINO)-4-OXOBUTANOIC ACID; *N*-DIMETHYLAMINOSUCCINAMIC ACID

COMMENTS AND RESPONSE TO COMMENTS ON CLH: PROPOSAL AND JUSTIFICATION

The proposal for the harmonised classification and labelling (CLH) of daminozide (ISO); 4-(2,2-dimethylhydrazino)-4-oxobutanoic acid; N-dimethylaminosuccinamic acid EC 216-485-9; CAS 1596-84-5) was submitted by Czech Republic and was subject to a consultation, from 24/07/2019 to 24/09/2020. The comments received by that date are compiled in Annex 2 to the opinion.

New information was submitted to ECHA in relation to carcinogenicity and mutagenicity hazard classes. An ad hoc consultation was launched from 16/03/2020 to 30/03/2020 and the comments received are listed below.

ECHA accepts no responsibility or liability for the content of this table.

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
26.03.2020	Switzerland	Daminozide task force	Company-Manufacturer	1
Comment received				
The main points as to why both papers opened for this targeted PC are unreliable, are presented in the corresponding hazard class boxes. A more detailed assessment is provided in attachment. It has to be noted that although these papers might not have been considered through the ECHA process yet, these have been in the public domain for a long time and extensively discussed in the 2004 EFSA PPPR Opinion of daminozide. ECHA note – An attachment was submitted with the comment above. Refer to public attachment 2020-03-25 Daminozide TF response to targeted CLH consultation final.pdf				
RAC's response				
Your comment is noted.				

Date	Country	Organisation	Type of Organisation	Comment number
30.03.2020	Germany		MemberState	2
Comment received				
The newly submitted data for the second round of targeted public consultation provides additional evidence that would support the Carc. 1B classification of daminozide but not classification for mutagenicity.				
RAC's response				
Thank you for a clear position on the classification proposal.				

CARCINOGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
30.03.2020	Germany		MemberState	3

Comment received

The carcinogenicity study conducted by the U.S. National Cancer Institute (Campbell et al., 1978; Technical Report Series No. 83) provides further evidence for the evaluation of carcinogenic potential of daminozide in rodents (rats and mice).

Below is the comparison of tumour incidence between the Campbell et al. (1978) study and the carcinogenicity study of Johnson (1988) already evaluated during the first round of consultation.

1. Pituitary adenomas in female rats: inconclusive in both studies
• Johnson (1988): some indication of increasing trend with the test substance but no clear monotonic dose-response, e.g. the highest dose group of 10000 ppm having similar tumour incidence as the control group
• Campbell (1978): some indication of increasing trend but also no clear dose-response trend in the incidence of pituitary (chromophobe) adenoma (3/19 in control, 7/45 in low dose of 5000 ppm and 8/43 in high dose of 10000 ppm)
In addition, there is no historical control data provided in the report for this tumour type, but existing data (e.g. from the U.S. NTP's historical control database; https://ntp.niehs.nih.gov/data/controls/index.html) reported an incidence for pituitary adenomas of over 40% in control Fischer 344 female rats from oral feed studies. Furthermore, the incidence of control group is not directly comparable with the treated groups given the lower number of animals used (see further below for study limitations).
2. Uterine/endometrium tumours in female rats: limited evidence; discrepant results from the 2 studies
• Johnson (1988): No treatment-related findings
• Campbell (1978): Slight increase in leiomyosarcoma in the uterus and endometrium adenocarcinoma, which were above the historical control incidence and also considered to be rare tumours for Fischer 344 female rats; however, low in incidence and no clear dose-response trend observed
3. Alveolar/bronchiolar adenomas + carcinomas in mice (both sexes): Some evidence of treatment-related increase in these tumours
• Johnson (1988): Inconclusive in male CD-1 mice but some evidence in female CD-1 mice
• Campbell (1978): Some indications of treatment-related increase in alveolar/bronchiolar adenomas and carcinomas in both male and female B6C3F1 mice but no historical control data reported for comparison
4. Hepatocellular carcinoma in male mice: limited evidence; discrepant results from the 2 studies
• Johnson (1988): No clear treatment-related findings
• Campbell (1978): Some indications of dose-related increase in hepatocellular carcinoma in male B6C3F1 mice; incidence reported to be higher than historical control data

The following key limitations in the Campbell (1978) study should be taken into consideration for the evaluation:
• lower number of control animals examined (20 instead of 50 examined per test substance concentration)
• no determination of daily intake concentration of test substance (e.g. in mg/kg bw/d)
• potential decomposition of the test substance in feed, as stated in the study report "The

recovery of test chemical from feed decreased about 20% after the feed was stored for 10 days at 25°C; thus, some decomposition of the test chemical appeared to occur in the feed hoppers under the conditions of this bioassay."
• lack of historical control data presented in the report (only briefly mentioned for the relevant tumours)

Altogether, there is sufficient evidence for carcinogenic potential of daminozide in laboratory animals with moderate degree of uncertainty, e.g. limitations of both carcinogenicity studies (mentioned in the first commenting round and here) as well as lack of understanding of the mode of action of daminozide leading to the formation of these tumours. It still remains unclear if UDMH (metabolite of daminozide with the harmonised classification as Carc. 1B) has a putative role in the tumour formation from exposure to daminozide.

Nevertheless, considering the existing data of tumours found in multiple organs and species and no further evidence to demonstrate the lack of human relevance, we would support the proposed Carc. 1B classification of daminozide.

RAC's response

Your detailed comparative analysis of both studies is highly appreciated. With respect to the pituitary adenomas in female rats reported in Johnson (1988), please refer to the RAC responses in the previous commenting round. Considering the high background incidence of this tumour type in Fischer rats, and the lack of clear dose-trend/statistical significance, RAC did not include the findings on pituitary adenomas from the Campbell et al. (1978) study in the overall assessment for this tumour type. RAC further notes that the incidence rates of uterine/endometrium tumours in female rats from the Campbell et al. (1978) study lack statistical significance, however these are rare tumour types with low background rates that should not be disregarded as a pure chance finding. RAC does not find further support for carcinogenicity classification based on the incidences of alveolar/bronchiolar adenomas and carcinomas, and hepatocellular carcinoma in B6C3F1 mice as reported in Campbell et al. (1978). From the documented limitations of the study, the most significant impact on the study outcome would be the possible formation of decomposition products that could potentially cause the observed uterine tumours. Unfortunately, there is no further data on the identity and quantity of these products. Considering the findings from the three carcinogenicity assays and their limitations, RAC concludes in an overall weight of evidence assessment that Category 2 is more appropriate.

Date	Country	Organisation	Type of Organisation	Comment number
28.03.2020	Belgium	Certis Europe BV	Company-Downstream user	4

Comment received

The document provided in attachement detailed the arguments for a proposal of Category 2 carcinogen.
In the regulatory cancer studies performed with daminozide a statistically significant increase in the overall incidence of pituitary adenoma in the female rat and of pulmonary tumours in male and female mice was reported at some dose levels.
A thorough analysis of the results of the regulatory cancer studies in rats and mice indicated that the CLP criteria for Category 1B (sufficient evidence of carcinogenicity) are not met and therefore there is not sufficiently convincing evidence to place the substance in Category 1B (presumed human carcinogen).

It is proposed to consider daminozide as a Category 2 carcinogen (suspected human carcinogen) according to the CLP criteria (Regulation (EC) No. 1272/2008).

ECHA note – An attachment was submitted with the comment above. Refer to public attachment Comments to the proposed classification of daminozide - Certis Europe BV - March 28th 2020.docx

RAC's response

Thank you for your comments. With respect to the increased incidence of pituitary adenomas, RAC considered additional information provided by the applicant that explains the very high terminal rates in female rats of the intermediate doses (100%), and does not include this tumour type as evidence supporting classification for carcinogenicity. RAC agrees that in an overall weight of evidence assessment category 2 is more appropriate.

Date	Country	Organisation	Type of Organisation	Comment number
26.03.2020	Switzerland	Daminozide task force	Company-Manufacturer	5

Comment received

Campbell et al., 1978 :
This early (1978) study in the NTP carcinogenicity testing programme, conducted with both F344 rats and B6C3F1 mice, was part of the data assessed in the 2004 EFSA PPPR Opinion of daminozide. No GLP or OECD test guidelines were in force at the time the study was conducted.
Under the conditions of this NTP study, daminozide was not carcinogenic in male Fischer 344 rats or in female B6C3F1 mice. In the male B6C3F1 mice, the apparent distribution of hepatocellular carcinomas would appear attributable to a low incidence in controls; this control group was compromised in any case by 6/20 (30%) animals missing, leaving a group size of only 14 mice. Daminozide appeared to be carcinogenic in female Fischer 344 rats, inducing adenocarcinomas of the endometrium of the uterus and leiomyosarcomas of the uterus; however since daminozide degradation in diet was not adequately managed the tumour response in either species cannot be definitively attributed to daminozide. Uterine tumours in female F344 rats were not repeatable under more appropriate test conditions in the GLP- and guideline-compliant study reported in 1988.

Due to the unsuitability of test diet stability, a Klimisch reliability score of 4 is appropriate. Unexplained loss of 6 from 20 male control mice also suggests poor animal care and management.

The daminozide task force recommends the 2004 EFSA PPPR Opinion which declined to rely on the NTP data:
"In the more recent studies conducted according to Good Laboratory Practice (GLP) standards (author redacted, 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." (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.)

ECHA note – An attachment was submitted with the comment above. Refer to public attachment 2020-03-25 Daminozide TF response to targeted CLH consultation final.pdf

RAC's response

Thank you for your analysis. RAC agrees that the unusualy low incidence of hepatocellular carcinomas in the control animals and their low number in this group may have affected the reported tumour distribution, therefore this tumour type was not considered as evidence supporting classification for carcinogenicity. It is further noted that a possible formation of degradation products that could potentially lead to formation of the uterine tumours in rats cannot be excluded. However, carcinogenicity studies with UDMH, a major metabolite/hydrolysis product of daminozide, did not show any evidence for this tumour type. Therefore, the Campbell et al. (1978) study is assessed in an overall weight of evidence approach, considering all its limitations. RAC is further aware of the previous assessment of these studies, and a reference is included in the opinion document.

MUTAGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
26.03.2020	Switzerland	Daminozide task force	Company-Manufacturer	6

Comment received

CLIET et al., 1989
The daminozide task force supports a conclusion that neither daminozide nor UDMH are genotoxic.

Cliet et al. 1989 describes the investigation of UDMH and NDMA genotoxicity by means of a mouse liver micronucleus (MN) test and was included in the EFSA PPPR opinion on daminozide (EFSA Journal (2004), 61, 1-27). There are significant deficiencies in this study that limit its reliability for assessment of UDMH genotoxicity. The liver MN endpoint described has been insufficiently validated, the results have not been independently replicated and a physiologically irrelevant route of administration has been used (intraperitoneal injection) that is no longer recommended for genotoxicity testing. However, the most critical deficiency is a lack of characterisation of the UDMH and NDMA used: no batch numbers or purity information are reported and UDMH was formulated in water with no indication of any measures taken to limit oxidation or pH-dependent degradation. Concerns regarding the reliability of results using "unprotected" aqueous formulations of UDMH were highlighted in the EFSA PPPR opinion. Cliet et al. shows clear evidence of confounding degradation of UDMH to NDMA. The MN frequency induced by UDMH is not dose-related and is comparable to the NDMA response, despite NDMA being tested at 10-fold lower doses. In contrast, in the daminozide task force submitted UDMH studies (where NDMA was used as the +S9 positive control) NDMA produced >6x more HPRT mutants (Stankowski 1988) and 4x more aberrations (San Sebastian 1986) than UDMH at 1/50th of the maximum UDMH concentration. The Cliet et al. reported findings for UDMH are likely to be due to the presence of a mutagenic degradation product (i.e. NDMA) rather than direct UDMH-DNA reactivity. In conclusion, Cliet et al. 1989 is unreliable (Klimisch reliability score 4 due to unsuitable test conditions ie instability of the tested formulation) as it provides inadequate characterisation of the UDMH batch, the UDMH formulation was not protected from oxidation/pH-dependent degradation and

an irrelevant route of exposure was used. The data generated are inconsistent with results obtained in all of the daminozide task force in vitro studies (which included protective measures to limit UDMH oxidation prior to cell exposure).

ECHA note – An attachment was submitted with the comment above. Refer to public attachment 2020-03-25 Daminozide TF response to targeted CLH consultation final.pdf

RAC's response

RAC agrees that the micronucleus test in mouse liver cells as reported by Cliet et al. (1989) is not a standardized guideline test in the context of REACH and CLP regulation. It is further noted that information on the stability of the test solution and on the formation of potential degradation products is not available. With respect to the application route, the authors justify the use of i.p. injection as a means to avoid a direct passage to the liver and a potential first-pass effect there. It is further noted that the response for both substances were comparable with these for other well-known mutagens examined in the same study, i.e., diethyl-nitrosamine (DEN), 4-aminophenol (4-APOL), 4-aminobiphenyl (4-ABPYL) and β-propiolactone (BPL). Together with the other studies provided in the registration dossier, RAC considers the results from Cliet et al. (1989) relevant for the in vivo genotoxicity assessment of UDMH and NDMA.

Date	Country	Organisation	Type of Organisation	Comment number
30.03.2020	Germany		MemberState	7

Comment received

The in vivo study of Cliet et al. (1989) demonstrates the clastogenic potential of UDMH (metabolite of daminozide) in mouse hepatocytes. However, this additional study, combined with the existing genotoxicity studies of daminozide, does not provide sufficient evidence that warrant classification for mutagenicity of daminozide for the following reasons:

1. Even though increased number of micronucleated hepatocytes was observed in adult male CD-1 mice treated with two daily i.p. administrations of UDMH (or 1,1-dimethylhydrazine/1,1-DMH as reported in the study) at all 3 tested doses (14, 28, and 56 mg/kg), no dose-response relationship was observed.
2. In vitro and in vivo studies on genotoxic potential of daminozide reported negative results. In particular, the combined in vivo micronucleus and chromosome aberration assay (performed according to OECD Guidelines 474 and 475) observed no increase in the incidence of chromosomal aberrations or micronucleated polychromatic erythrocytes in ICR mice of both sexes exposed i.p. to daminozide (500-2000 mg/kg for males and 375-1500 mg/kg for females; proven bone marrow exposure via the reduction in the proportion of immature erythrocytes among total erythrocytes).
3. There is insufficient information on the in vivo metabolism of daminozide to UDMH in mice or humans (as already mentioned above for carcinogenicity)

RAC's response

Thank you for your analysis. RAC agrees that within the scope of the existing genotoxicity studies on daminozide, the results from Cliet et al. (1989) demonstrating a clastogenic potential of UDMH in mouse hepatocytes do not provide sufficient evidence for mutagenicity classification of daminozide. Nevertheless, the study is assessed as a part of the (geno)toxicity profile of UDMH, a putative metabolite of daminozide. It is further noted, that while a clear dose trend in the number of micronucleated cells is lacking, the response was comparable to other well-known mutagens examined in the same study. The formation of potentially genotoxic/carcinogenic metabolites is substantial part of the weight of evidence that should be considered for carcinogenicity classification.

ANNEX 3 RECORDS OF THE TARGETED PUBLIC CONSULTATION ON DAMINOZIDE (ISO); 4-(2,2-DIMETHYLHYDRAZINO)-4-OXOBUTANOIC ACID; *N*-DIMETHYLAMINOSUCCINAMIC ACID

PUBLIC ATTACHMENTS

1. Comments to the proposed classification of daminozide - Certis Europe BV - March 28th 2020.docx [Please refer to comment No. 4]
2. 2020-03-25 Daminozide TF response to targeted CLH consultation final.pdf [Please refer to comment No. 1, 5, 6]