Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

reaction mass of 1-[2-(2-aminobutoxy)ethoxy]but-2-ylamine and 1-({[2-(2-aminobutoxy)ethoxy]methyl}propoxy) but-2-ylamine

EC Number: 447-920-2
CAS Number: -

CLH-O-0000001412-86-132/F

Adopted

9 December 2016

9 December 2016

CLH-O-0000001412-86-132/F

OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL

In accordance with Article 37 (4) of Regulation (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonised classification and labelling (CLH) of:

Chemical name:	**reaction mass of 1-[2-(2-aminobutoxy)ethoxy]but-2-ylamine and 1-({[2-(2-aminobutoxy)ethoxy]methyl} propoxy)but-2-ylamine**
EC Number:	**447-920-2**
CAS Number:	**-**

The proposal was submitted by **Belgium** and received by RAC on **4 February 2016.**

In this opinion, all classification and labelling elements are given in accordance with the CLP Regulation.

PROCESS FOR ADOPTION OF THE OPINION

Belgium has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **7 March 2016**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **21 April 2016**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Bert-Ove Lund**

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation and the comments received are compiled in Annex 2.

The RAC opinion on the proposed harmonised classification and labelling was adopted on **9 December 2016** by **consensus**.

Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	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	612-RST-00-Y	reaction mass of 1-[2-(2-aminobutoxy)ethoxy]but-2-ylamine and 1-({[2-(2-aminobutoxy)ethoxy]methyl}propoxy)but-2-ylamine	447-920-2	NA	Acute Tox. 4 Skin Corr. 1B Eye Dam. 1 Repr. 2	H302 H314 H318 H361fd	GHS05 GHS07 GHS08 Dgr	H302 H314 H361	-	-	-
RAC opinion	612-RST-00-Y	reaction mass of 1-[2-(2-aminobutoxy)ethoxy]but-2-ylamine and 1-({[2-(2-aminobutoxy)ethoxy]methyl}propoxy)but-2-ylamine	447-920-2	NA	Acute Tox. 4 Skin Corr. 1B Eye Dam. 1 Repr. 2	H302 H314 H318 H361f	GHS05 GHS07 GHS08 Dgr	H302 H314 H361f	EUH071	-	-
Resulting Annex VI entry if agreed by COM	612-RST-00-Y	reaction mass of 1-[2-(2-aminobutoxy)ethoxy]but-2-ylamine and 1-({[2-(2-aminobutoxy)ethoxy]methyl}propoxy)but-2-ylamine	447-920-2	NA	Acute Tox. 4 Skin Corr. 1B Eye Dam. 1 Repr. 2	H302 H314 H318 H361f	GHS05 GHS07 GHS08 Dgr	H302 H314 H361f	EUH071	-	-

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

The substance to be classified is a multiconstituent substance, defined as the reaction mass of 1-[2-(2-aminobutoxy)ethoxy]but-2-ylamine and 1-({[2-(2-aminobutoxy)ethoxy]methyl} propoxy)but-2-ylamine (trade name XTJ-568), roughly containing the two components in a 3:1 ratio. This polyetherdiamine is highly soluble in aqueous solutions, resulting in a basic pH. However, for the reproductive toxicity studies, a dihydrochloride salt of the substance was used, which dissociates in water to form the free diamine.

The substance to be classified will be referenced throughout the document by its trade name, XTJ-568.

The CLH proposal addressed six endpoints; although other endpoints were also commented on during the public consultation, this opinion only covers those proposed by the dossier submitter.

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

Two acute toxicity studies via the oral route revealed LD_{50} values between 300 and 2000 mg/kg bw (1000 mg/kg bw for the first study and between 200 and 2000 mg/kg bw for the second study). The acute oral toxicity of XTJ-568 thus fulfils the CLP criteria for classification in category 4, H302 (harmful if swallowed).

Based on the results of a dermal acute toxicity study showing an LD_{50} > 2000 mg/kg bw, the DS proposed no classification for the dermal route of exposure.

There were no acute inhalation data.

Comments received during public consultation

No comments were received in relation to acute toxicity.

Assessment and comparison with the classification criteria

RAC notes that mainly female rats were studied in the acute oral studies. Although using only females for the testing is appropriate according to the test guidelines, the absence of data on male rats (other than the information that 3 male rats survived treatment with 200 mg/kg bw) and the small number of animals tested introduces some uncertainty to the assessment. Two out of three female rats died at 1000 mg/kg bw and 2000 mg/kg bw in the two studies, respectively, and none in the next lower doses (550 and 200 mg/kg bw, respectively). Based on these two studies, the oral LD_{50} in female rats seems to be below 1000 mg/kg bw, but above 550 mg/kg bw. Overall, the data support an LD_{50} value in the range of 300-2000 mg/kg bw for oral acute toxicity and RAC therefore concludes that the substance should be classified as **Acute Toxicity 4 via the oral route, H302**.

A limit dose study was performed for the dermal route (2000 mg/kg bw) and although 2 out 5 females died, all males survived, resulting in an LD_{50} > 2000 mg/kg bw for the dermal route, and thus RAC agreed with the DS that **no classification for the dermal route** was warranted.

Due to the substance being corrosive to the skin, Industry waved the acute toxicity inhalation studies (Annex VII of the Reach Regulation). RAC agrees with the DS conclusion that no classification for the inhalation route of exposure was possible, since there was no data available.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

In an OECD TG 404 rabbit study, erythema was observed after 3 min of exposure to the pure substance. After 1 hour of exposure, effects became more severe with a full thickness destruction of the skin (wounds with serious exudate, grey discolouration, signs of necrosis), and the initially treated rabbit was humanely sacrificed after the 1 hour exposure period. Therefore the DS proposed that the substance should be classified for skin corrosivity, category 1B, H314 (causes severe skin burns and eye damage).

Comments received during public consultation

No comments were received.

Assessment and comparison with the classification criteria

As erythema, but no necrosis, was observed after 3 minutes exposure, category 1A is not appropriate. However, full thickness destruction of skin, wounds with serious exudate, grey discoloration and sign of necrosis were observed in the single exposed rabbit after 1 hour exposure, fulfilling the criteria for category 1B.

In addition, it is noted that dissolving XTJ-568 in aqueous media will result in a basic pH (pH 12.5 at 500 g/L), likely explaining the corrosivity observed in the rabbit and further supporting classification. Thus, RAC agrees with the proposal of the DS for classification for **skin corrosivity, 1B, H314 (causes severe skin burns and eye damage)**.

The dossier submitter did not address further labelling with EUH071 and RAC notes that there is no inhalation toxicity data available for XTJ-568. According to CLP (Article 25.1 and Annex II: 1.2.6), corrosive substances shall be labelled 'EUH071, Corrosive to the respiratory tract' if there is no acute inhalation toxicity data available and the substance may be inhaled. The substance is a liquid with low vapour pressure (3.4 Pa at 20°C) which decomposes at 148°C, before boiling. Given the physico-chemical nature of this substance and the possibility that it may be inhaled, RAC proposes additional labelling with **EUH071 (Corrosive to the respiratory tract)**.

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

There were no test data for eye damage or irritation. The DS noted that according to CLP, a skin corrosive substance is also considered to cause serious eye damage, which is indicated in the

hazard statement for skin corrosion (H314: causes severe skin burns and eye damage). Thus, in this case both classifications (Skin Corr. 1B and Eye Dam. 1) are required but the hazard statement under labelling "H318, causes serious eye damage" is not needed on the label because of redundancy.

Comments received during public consultation

No comments were received.

Assessment and comparison with the classification criteria

There is no specific data on eye irritation/corrosion, but RAC agrees that eye corrosion can be expected.

However, eye corrosion is covered by the proposed classification for skin corrosion (category 1B, H314 (causes severe skin burns and eye damage). RAC proposes, in line with the Commission clarifications, that there should be additional classification with **Eye Dam. 1, H318**, but no labelling (i.e. no use of H318 "Causes serious eye damage" under labelling).

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

An Ames test, two *in vitro* aberration tests and one *in vivo* mutagenicity study were conducted with XTJ-568. Some effects (increased number of polyploid cells) were seen in one *in vitro* mammalian chromosome aberration test. However, the substance was not considered by the DS to be a clastogen under the conditions of the test. As all other studies showed negative outcomes, overall the results were seen as being negative and XTJ-568 was not considered by the DS to be a germ cell mutagen.

Comments received during public consultation

Three MSCA commented that classification is not warranted. However, one commented that the lack of data made an independent assessment difficult, and another that the available database was not sufficient to evaluate the mutagenicity (no *in vitro* gene mutation test was available, there was no evidence that the substances had reached the bone marrow in the *in vivo* micronucleus assay) and that the no classification conclusion should therefore be based on lack of data rather than on the absence of mutagenic potential.

Assessment and comparison with the classification criteria

The substance was negative in the Ames tests and no chromosome aberrations were observed in two *in vitro* chromosome aberration tests. An increased number of polyploid cells was observed in one of the studies. In contrast, no statistically significant increase in the incidence of micronucleated polychromatic erythrocytes was observed in an *in vivo* mouse micronucleus assay. Although some uncertainty in evaluating the study was caused by there being no reduction in the PCE/EC-ratio, and thus no evidence that the substance reaches the bone marrow, clinical signs of lethargy and piloerection likely indicated systemic exposure to XTJ-568. Thus, based on lack of *in vivo* signs of mutagenicity, RAC agrees with the DS that **no classification is warranted for germ cell mutagenicity**.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

Fertility

In a 2-generation toxicity study in rats, treatment with XTJ-568 dihydrochloride resulted in a statistically significant decrease in the left epididymis weight (at the high dose, 1000 mg/kg bw/d). Microscopic examination showed, in the male high dose group, statistically significant minimal or slight multifocal seminiferous degeneration in the testis and minimal or slight seminiferous cell debris in the epididymis. The sperm evaluation confirmed these results and revealed a statistically significant decrease at 1000 mg/kg bw/d of sperm concentration in the left epididymis and left testis. The percentage of sperm cells with normal morphology was decreased and the number of sperm with separated head was increased (also observed at 450 mg/kg bw/d). A slightly lower sperm mobility was observed already at 150 mg/kg bw/d. The lack of effects on the testis in the repeated dose 13-week toxicity study at doses up to 150 mg/kg bw/d were not considered to negate those findings. Therefore classification for effects on fertility in category 2 was proposed (Repr. 2, H361f).

Developmental toxicity

In a standard OECD TG 414 developmental toxicity study, no effects were observed at up to the highest dose of 1000 mg/kg bw/d. However, delayed postnatal development was observed in the 2-generation study, expressed as a statistically significant delay in balanopreputial separation and vaginal opening in the F1 generation. The anogenital distance was significantly lower in males at 150 and 450 mg/kg bw/d and in females at 150 mg/kg bw/d in the F2 generation. A classification for developmental toxicity in category 2 was therefore proposed (Repr. 2, H361d).

The overall reproductive toxicity classification, proposed by the dossier submitter, was Repr. 2, H361fd.

Comments received during public consultation

Comments were received from 2 MSCAs and one Industry organisation. They were all supportive of classification in Cat. 2 for effects on fertility, but questioned classification for developmental toxicity on the basis that the effects on development were considered to be most likely caused by a slower growth of the pups.

Assessment and comparison with the classification criteria

Fertility

For effects on fertility, the 2-generation study is the key study and effects on the testis the key toxicological effect. At the top dose (1000 mg/kg bw/d), salivation (perhaps caused by bad taste of XTJ-568) and a statistically decreased body weight gain were observed. It was very difficult to get a clear picture about the magnitude of the decreased weight gain. Palatability may have been an issue, as indicated by slightly lower food consumption, but the extent was not clear. In F0 adults, the decreased body weight gain seemed less than 10%, and if so, this constituted only limited general toxicity.

Substantially decreased body weights (-28%) were observed in male top dose F1 pups (of unclear age, perhaps day 21) and 18% lower body weight at necropsy of the adult F1 males. In F1 female

the effects on body weight at the top dose were lower (-22% and -5%, for pups and adults respectively). At the mid dose (450 mg/kg bw/d), the F1 body weights were decreased by < 8%.

Testis-related findings in the F0 generation

1000 mg/kg bw/d

In the CLH dossier, minimal or slight multifocal seminiferous epithelial degeneration in testis and minimal or slight seminiferous cell debris in epididymis in 3 animals at the top dose ($p \leq 0.01$) were reported. Other findings at the top dose included a statistically significant decrease in absolute left epididymis organ weight (possibly caused by the decreased body weight; relative weight not given), a decreased sperm concentration of the left epididymis (-29%; $p < 0.01$) and left testis (-13%; but not statistically significant), decreased sperm motility score of 2 (vs 3 in controls), and decreased percentage of sperm cells with normal morphology (63% vs 87% in the controls).

450 mg/kg bw/d

Some effects were also observed at the mid dose, including a statistically significant decreased sperm concentration of the left epididymis (-22%; $p < 0.05$) and left testis (-19%; $p < 0.05$), decreased sperm motility score (2 vs 3 in controls), and decreased percentage of sperm cells with normal morphology (63% vs 87% in controls).

Testis-related findings in the F1 generation

1000 mg/kg bw/d

Upon macroscopic examination, 3 males in the top dose group showed reduced size of testis, epididymides and/or seminal vesicles together with oligospermia in the epididymis, multifocal seminiferous epithelial degeneration in the testis or hypotrophic acini in the seminal vesicles. Other findings at the top dose included statistically significant decreases in absolute weight of the epididymis, testes, prostate, and seminal vesicles (likely affected by the decreased body weight; relative weights not given). Microscopic examination revealed minimal multifocal seminiferous epithelial degeneration and minimal seminiferous cell debris in 5 top dose animals vs 1 in controls. The sperm examination showed a statistically significant decrease in sperm concentration (-32%) in the left epididymis and sperm quality (motility was decreased; 30% vs 54% in controls), progressive motility (13 vs 28% in controls), and low percentage of sperm cells with normal morphology (64 vs 92% in controls). The litter size was decreased (7.9 pups/litter vs 11.1 in the controls). According to information received during the Public Consultation (Huntsman BVBA, see the RCOM), fewer implantation sites were also observed in the group with the decreased litter size, perhaps supporting a relationship to the testis toxicity. However, similar effects were not observed in F0 animals.

450 mg/kg bw/d

The sperm examination revealed a statistically significant decrease in percentage of sperm cells with normal morphology (65% vs 92% in controls). The other sperm parameters were decreased (by 8%-17%), but the decreases were not statistically significant.

In a range-finding 28 day study in Wistar rats, all animals in the 1000 mg/kg bw/d dose group were sacrificed before the end of the study because of extensive toxicity. On the other hand, rather limited toxicity was observed in Wistar rats exposed to 1000 mg/kg bw/d during days 6-19 in a developmental toxicity study (no statistical effects on body weight gain but some clinical signs) and in Wistar rats of the 2-generation study (effects on body weight gain). In these three studies showing very different levels of toxicity, the same strain of rats was used and all involved gavage exposure. However, in the 2-generation and developmental toxicity studies with low general toxicity in adult rats, the substance was administered as a dihydrochloride salt of XTJ-

568, whereas another batch of XTJ-568 (non-salt) was used for the range-finding 28 day (Anonymous 22, 2003) repeated dose toxicity study where extensive toxicity was observed (at 1000 mg/kg bw/d). This may indicate that the inherent toxicity of the salt vs the non-salt differs, which is most likely related to the pH of the dosing solution and the rate of release of the free diamine. The chemical composition of the different batches of substance is otherwise not expected to differ (see RCOM; Huntsman BVBA). Although differences in toxicity were indicated by the available studies, it is generally considered acceptable to perform studies using the salt of a substance (vs pure substance) to avoid problems with pH, and RAC is therefore of the opinion that the above developmental toxicity and 2-generation studies can be used to assess the reproductive toxicity of XTJ-568.

Repeated dose toxicity studies usually provide additional information on testes toxicity, but the available studies for this substance used lower exposure levels (≤ 150 mg/kg bw/d) than the 2-generation study, therefore the lack of testicular toxicity in those studies did not contradict the findings in the 2-generation study.

The 2-generation study showed a relatively consistent picture of testicular toxicity between the generations. The major effects on body weight in the F1 animals was a concern when evaluating the study, but the findings in F0 animals and the indications in the mid dose of F1 occurred without concomitant effects on body weights. Although it could be a possible borderline case, RAC is of the view that there is sufficient evidence to classify based on direct effects on the testes caused by XTJ-568.

As no human data is available, category 1A is not relevant. Category 1B can be applied when animal studies provide clear evidence of effects on fertility. However, there are some uncertainties arising from maternal toxicity occurring in the F1 generation at the top dose, effects mainly occurring at very high exposure levels which may be not be easily encountered for a substance with such a high pH, and the fact that the 2-generation study has been performed on a salt of XTJ-568 and not the pure substance. Still, the 2-generation study is considered to provide "some evidence" of effects on fertility, thus **RAC concludes that classification in category 2 (H361f)** is warranted.

The CLH report did not propose setting a specific concentration limit (SCL), but RAC notes that based on the available data (ED_{10} > 400 mg/kg bw/d) the substance could be considered to belong to the low potency group, where an SCL of 3-10% could be considered. The generic concentration limit (GCL) for category 2 is 3%, but based on the CLP guidance there is not sufficient reason for deviating from the GCL in this case.

Developmental toxicity

Significantly delayed balanopreputial separation and delayed vaginal opening in the F1 generation by 2 and 1 days, respectively, were observed in the 2-generation study. However, similar delays were not reported for F2 pups. In contrast, whereas no effects on anogenital distance was reported for F1 pups, significantly lower anogenital distance values were reported in F2 pups at 150 (both sexes) and 450 mg/kg bw/d (males). No effects were found in the top dose animals. At the top dose in F1 and F2 pups, decreased body weights were observed during the latter part of the weaning period. Because of lack of consistency between the generations, small effects, and likely correlation to lower body weight gain, RAC is of the opinion that the observed effects are not sufficiently adverse according to the criteria to warrant classification for developmental toxicity.

RAC concludes that the resulting overall classification for reproductive toxicity should be **Repr. 2, H361f**.

ENVIRONMENTAL HAZARD EVALUATION

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

There is currently no entry for XTJ-568 in Annex VI of CLP, but when the substance was evaluated in 2004 under the Dangerous Substances Directive (Dir. 67/548/EEC), an environmental hazard classification (R52/53) was proposed by the evaluating MSCA. New aquatic toxicity tests and degradation studies have since been conducted, requiring a new assessment of the need for classification for environmental hazards.

A ready biodegradability test (OECD TG 301B) and an inherent biodegradability test (OECD TG 302C) both showed that XTJ-568 is not readily biodegradable, with only a few percent degradation within 28 days. An experimentally derived log K_{OW} of 2 (OECD TG 117) indicated a low potential for bioaccumulation. Acute and chronic aquatic toxicity tests were available for all trophic levels, with the lowest EC_{50} being 88 mg/L and the lowest NOEC 4.6 mg/L, both being above the criteria for classification. The DS concluded that the substance should therefore not be classified for environmental hazards.

Comments received during public consultation

One MSCA commented and agreed with the proposal for no classification for environmental hazards.

Assessment and comparison with the classification criteria

XTJ-568 is hydrolytically stable.Reliable ready and inherent degradability tests (OECD TG 301B and 302C, respectively) show only a few percent mineralisation within 28 days, leading to the conclusion that the substance is not rapidly degradable.

There is no measured BCF available, but the measured log K_{OW} was 2 (OECD TG 117), indicating a low potential for bioaccumulation.

There are acute and chronic toxicity data for fish (*Cyprinus carpio, Danio rerio*), invertebrates (*Daphnia magna*), as well as a 72h static algal growth inhibition test (*Pseudokirchneriella subcapitata*). In many tests, the EC_{50}/NOEC were higher than the highest tested concentrations. Some of the chronic studies were not conducted according to GLP, and this may decrease their reliability. However, RAC evaluated the available information and concluded that the studies could still been used in the assessment. For acute toxicity, *Daphnia magna* was the most sensitive species with a 48h EC_{50} of 88 mg/L (nominal). For chronic toxicity, the most sensitive species was the algae *Pseudokirchneriella subcapitata (former Selenastrum capricornutum)* with a 72h NOE_rC = 4.6 mg/L (nominal).

Classification for acute aquatic toxicity would be relevant if the EC_{50} had been < 1 mg/L, and since the lowest EC_{50} is 88 mg/L, RAC agrees with the DS that **no classification for acute aquatic toxicity** is warranted.

For a substance which is not rapidly degradable and which has chronic toxicity data available for all three trophic levels, classification for chronic aquatic toxicity would be relevant if the NOEC would be < 1 mg/L. Since the lowest NOAEC is 4.6 mg/L, RAC concludes that XTJ-568 **should not be classified for chronic aquatic toxicity**.

ANNEXES:

Annex 1 The Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the Dossier Submitter; the evaluation performed by RAC is contained in 'RAC boxes'.

Annex 2 Comments received on the CLH report, response to comments provided by the Dossier Submitter and RAC (excluding confidential information).