Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

to the Opinion proposing harmonised classification and labelling at EU level of

Cyanamide; Carbamonitril

EC Number: 206-992-3
CAS Number: 420-04-2

CLH-O-0000001412-86-67/F

Adopted
5 June 2015

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON CYANAMIDE

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 attachments including confidential documents received during the public consultation have been provided in full to the dossier submitter, to RAC members and to the Commission (after adoption of the RAC opinion). 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.

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Substance name: Cyanamide; Carbamonitril
CAS number: 420-04-2
EC number: 206-992-3
Dossier submitter: Germany

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
07.11.2014	France		MemberState	1
Comment received				
MS FR agrees for the classification proposal based on the data reported in the CLH report for environmental hazards. However in the biocide dossier, a water sediment study, showing more than 70% of mineralisation in 28 days, is available and could support that the substance is rapidly biodegradable (point c and d in the paragraph 4.1.3.2.3.2 from the Guidance on the Application of the CLP criteria). Could the MS-DE explain why this study has not been taken into account for the classification? Please, the minimum purity should be clarified in all CLH report. Based on the manufacturing process, it seems that the substance can be used directly under form of aqueous cyanamide solution consequently; the purity of this solution should also be reported as minimum purity.				
Dossier Submitter's Response				
1. Part: Thank you for the comment. We did not consider the water-sediment study as we interprete point b and c as referring to a water simulation study (OECD 309) which is not available. Our understanding of the decision scheme is that a water-sediment simulation study (point d) is only considered if none of the studies mentioned under point a-c is available. For this reason we used the ready biodegradability study for the decision only. However, as in the water-sediment study nearly most of the radioactivity was found in the water phase in a weight of evidence consideration of the water-sediment study is possible and cyanamide can be considered as rapidly degradable. As the observed metabolites except urea are below 10% they need not to be considered. For the major metabolite urea (max. 13.4 % in pond system) DT_{50} values of 5.5 days (river) and 15.2 days (pond) were determined. Thus, urea can also be considered as rapidly degradable.				
RAC's response				
Noted. RAC has taken account of the water sediment study and agrees with FR that the results support rapid biodegradability.				

Date	Country	Organisation	Type of Organisation	Comment number
10.11.2014	Spain	FAES FARMA, S.A.	BehalfOfAnOrganisation	2

Comment received

FAES FARMA, S.A. is the Marketing Authorization Holder of Colme®, a cyanamide containing drug used in the alcohol aversion therapy.
Colme® is authorised in Spain since 1971. Subsequently, the product was registered in Russia (1999), the Ukraine (2006) and Mongolia (2011).
Based on our vast human experience with cyanamide we would like to make the following comments:

1. Identity of active ingredient of Colme
As there is often confusion in the literature regarding the active ingredient of Colme we would like to make clear that Colme is an aqueous solution containing (hydrogen) cyanamide and NOT calcium cyanamide. Therefore please correct the statement made on page 219 (Conclusion) to "It is reported that calcium cyanamide and hydrogen cyanamide are used as effective deterrents to alcohol consumption because they …" and the statement made in Chapter 4.12.2 Summary and discussion to "Calcium cyanamide and hydrogen cyanamide have been worldwide intensively used as a drug to deter drinking in alcoholics".

2. Cyanamide in alcohol therapy (page 214 to 222 of the CLH report)
Based on an average dose of 1 mg/kg bw/d hydrogen cyanamide (60 mg per person per day) and the mean treatment duration of three months (90 days) the number of treated patients was estimated as follows:
As stated in the CLH report, the number of patients treated with Colme was 722,263 in the 10-year period from 1996 to 2006. From 2006 onwards about 250,000 patients have been treated annually. Thus, in addition to the previous experience, during the last 8 years (2006-2014) additional 2 million patients have been treated with Colme.
This clearly shows that a vast human database exists for hydrogen cyanamide which needs to be taken into account for the harmonised classification of this substance.

3. Adverse reactions given in the PSRs (page 220 and 221 of the CLH report and STOT RE, page 104 ff)
Generally, approximately 15 % of all hydrogen cyanamide treated patients may experience some minor side effects such as fatigue, dizziness and loss of appetite. Such effects are generally mild and disappear without having to discontinue treatment.
In contrast to the a.m. side effects, hypothyroidism in hydrogen cyanamide treated patients is considered rare (>1/10000; <1/1000).
During the 18-year period from 1996 to 2014 no case of adverse effects on the thyroid has been reported to our company nor have we found any case of adverse thyroid effects reported in the more recent literature.

Dossier Submitter's Response

It is noted that Colme is an aqueous solution containing (hydrogen) cyanamide.

It is noted that there is a vast human database for hydrogen cyanamide with respect to the worldwide use as a drug to deter drinking in alcoholics. No case of adverse effects on the thyroid has been reported in these patients (Periodic safety reports by Colme and others in the CLH report (May 2014) and Additional Report Cyanamide – Annex B.6: Toxicology and metabolism, 4 January 2010).

Considering these data for a harmonised classification of cyanamide the following aspects

have also to be taken into account.
Alcoholics have generally severe organ health problems because of a long lasting alcohol abuse. Alcoholics are therefore not comparable to healthy persons in terms of physiological reactions with xenobiotica as cyanamide. Thus human data based on health reports of alcoholics in drug therapy with cyanamide are not representative for other population groups. On the other hand there are only insular epidemiological data from other population groups (CLH report May 2014). There are no comprehensive epidemiological data from other population groups including healthy people exposed to cyanamide that would allow the conclusion to exclude adverse effects on the thyroid in humans as a toxic effect of cyanamide. Furthermore the CLP criteria for classification of substances for STOT-RE are also based on the evidence from studies in experimental animals in both catagories.

RAC's response

The RAC agrees that the alcoholic population are not necessarily representative of the general population. The need for treatment with alcohol deterrent therapy infers chronic long term abuse which may be associated with other health issues. However, would alcoholism 'protect' from a potential antithyroid effect of cyanamide. This seems unlikely.

Date	Country	Organisation	Type of Organisation	Comment number
10.11.2014	Germany	AlzChem AG	Company-Manufacturer	3

Comment received

First of all AlzChem wants to acknowledge the high quality of the CLH report prepared by the Dossier submitter. We fully agree with the assessment and recommendations made with the exception of the classification proposal for target organ toxicity and environmental hazards. Corresponding comments will be given under the respective hazard classes.

Dossier Submitter's Response

It is noted that AlzChem does not agree with the classification proposal for target organ toxicity. See our comment Number 2.

RAC's response

Noted.

CARCINOGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
04.11.2014	Netherlands		MemberState	4

Comment received

The NL CA disagrees with no classification for carcingenicity because:
• The incidence of benign and malignant phaeochromocytomas in rats was greater than the normal incidence (Table 106, p. 137 CLH Report).
• There was an increase in hemangiosarcomas in male mice and malignant lymphomas in female mice (Table 109, p. 139 CLH Report).
• There was a slight increase in ovarian granulosa-theca tumors observed in the high dose group females (200 and 600 mg/kg/day of hydrogen cyanamide; Table 116, p. 145 CLH Report).
We also do not agree with the arguments that carcinogenic effects above the MTD are by definition not relevant or that a comparison with the anticipated human exposure would affect the conclusion on classification.

Altoghether the data from findings in three carcinogenicity studies provide limited evidence for carcinogenicity and a classification of Carc. 2 (H351) should be considered. This is in line

with the EFSA (2010) conclusion on the peer review of the pesticide risk assessment of cyanamide.
Dossier Submitter's Response
Classification of cyanamid has been largely discussed in the CLH report (May 2014) based on data of three studies on carcinogenicity, two in mice and one with rats, and one long-term study with rats. In the chronic toxicity study with Sprague Dawley rats (Osheroff, M.R., 1991) and in the carcinogenicity study with F344 (Fischer) rats (Ulland, et al., 1979) no tumor at any site in the rats could be associated with the administration of the substance. In the carcinogenicity study with B6C3F1 mice (Ulland, et al., 1979) calcium cyanamide was not carcinogenic under the conditions of this study. In the carcinogenicity study with [Crl:CD-1 (ICR) BR] mice at the high dose (600 ppm corresponding to 39.0 mg/kg bw/day hydrogen cyanamide) in females a slight increase in granulosa-theca tumours was found (Goodyer, M.J., 1990). The Maximum Tolerable Dose (MTD) was exceeded at this dose level. It is concluded that no treatment related changes in the tumour profile were found up to a dose of 200 ppm (12.1 mg/kg/ bw day hydrogen cyanamide). Therefore cyanamide was not proposed to be classified as potential carcinogen CLH report (May 2014). No further data are available to evaluate the carcinogenic potential of cyanamide.
RAC's response
The RAC agrees that the tumours seen at dose near or in excess of the MTD also need to be considered in the light of the CLP criteria and agrees that there is significant indication of possible carcinogenicity on the basis of tumours in F344 rats and B6C3F1 mice.

Date	Country	Organisation	Type of Organisation	Comment number
07.11.2014	France		MemberState	5
Comment received				
MS FR doesn't comment on this endpoint.				
Dossier Submitter's Response				
noted				
RAC's response				
Noted.				

MUTAGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
04.11.2014	Netherlands		MemberState	6
Comment received				
The NL CA agrees for no classification for mutagenicity.				
Dossier Submitter's Response				
It is noted that NL CA agrees for no classification for mutagenicity.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment

				number
07.11.2014	France		MemberState	7
Comment received				
MS FR doesn't comment on this endpoint.				
Dossier Submitter's Response				
noted				
RAC's response				
Noted.				

TOXICITY TO REPRODUCTION

Date	Country	Organisation	Type of Organisation	Comment number
05.11.2014	Germany	AlzChem AG	Company-Manufacturer	8
Comment received				

4.11.2 Developmental toxicity:
Herewith we submit to the RAC a new prenatal developmental toxicity study (Pique, 2014) according to 440/2008/EC, B.31 and OECD 414 (2001). As this study may shed some further light on the relationship of developmental toxicity (especially diaphragmatic hernias) and maternal toxicity, this aspect will also be shortly discussed.
In the CLH report a review of Harris (2012) is mentioned (p. 190) who concluded that in the already submitted prenatal developmental toxicity study (Morseth, 1989) findings were mistakenly classified as diaphragmatic hernias and therefore proposed to conduct a new study. In addition the US authorities questioned the NOAEL of this study. Hence, AlzChem decided to commission a new prenatal developmental toxicity study according to the most recent OECD guideline.

This new study (Pique, 2014) basically had the same design with a similar dosing scheme as compared to the former study (Morseth, 1989). The most important differences were:
- Prolongation of dosing according to the current OECD guideline from GD 6 through GD 19.
- A low dose of 3 mg/kg bw/d instead of 5 mg/kg bw/d.
- A detailed categorization of the diaphragmatic hernias according to size, position and associated findings.

The most important findings in this study (Pique, 2014) were:
- Marked respectively severe maternal toxicity at 15 and 45 mg/kg bw/d of hydrogen cyanamide (similar to or even exceeding the corresponding effects in the Morseth study (1989)).
- 3 animals suffered such severe anorexia during the first 6 days of dosing that ethical sacrifice was considered. At 45 mg/kg bw/d adverse effects were recorded, especially diaphragmatic hernias (incidence similar to that in the Morseth study (1989)).
- All diaphragmatic hernias were small without impact on lung size.
- A clear NOAEL was established at 3 mg/kg bw/d.

The severe maternal toxicity observed in the studies of Pique (2014) and Morseth (1989) prompted a detailed assessment of foetal effects in relation to maternal toxicity (Gelbke, 2014). The definition of severe maternal toxicity given in the OECD guideline 414 (2001) for prenatal toxicity is relatively vague. Therefore, that given in the OECD guideline 426 (2007) for developmental neurotoxicity was applied.

This led to the conclusion that due to the extremely high maternal toxicity at 45 mg/kg

bw/d, the findings observed in this dose group should not be considered for developmental toxicity classification.

However, if the critical effect noted in the high dose groups of both studies (diaphragmatic hernias) is taken into account, the fact that for this effect the Dossier Submitter has demonstrated a specific maternally-mediated mechanism should be considered as well.

Therefore, a classification into Category 2 (H361d) could be suitable for reasons of precaution.

The following comparison of the developmental effects and the maternal toxicity of Hydrogen cyanamide further supports the classification proposal of the dossier submitter
Developmental effects and maternal toxicity of Hydrogen cyanamide
The following evaluation is based on two developmental toxicity studies conducted with Hydrogen cyanamide (HC) in Sprague Dawley rats. In one study (HLA 2319-124, Morseth, 1989) rats were treated with dose levels of 5, 15 and 45 mg/kg bw/d given from gestation day (GD) 6 through GD15. In the other study (AB17927, Pique, 2014) rats where treated with 3, 15 and 45 mg/kg bw/d, applied from GD 6 through GD 19. The second study was performed to verify or refute the induction of diaphragmatic hernias, to assign a systematic categorization according to size, and to gain further information about the severity/relevance of maternal toxicity for the effects found in the offspring and to establish a clear NAOEL.

The mid and high dose levels of the second study (Pique, 2014) were therefore selected solely to replicate the design of a previous prenatal developmental toxicity study in the Sprague-Dawley rat (Morseth, 1989) which had yielded equivocal results according to the assessment of Harris (2012). In the older study a moderate maternal toxicity in the mid dose and a marked maternal toxicity in the high dose were elicited. In the newer study these effects were even more pronounced particularly in the high dose group. Therefore, based on the principles of reproductive toxicology and today's knowledge regarding the toxicological properties of HC, the high dose for a developmental toxicity study would have been selected in the range of 15 mg/kg bw/day as this dose clearly meets the validity criteria of OECD Guideline 414 for a high dose setting.
The relevant OECD Guideline 414 (Prenatal Developmental Toxicity Study, 2001) states that "the highest dose should be chosen with the aim to induce some developmental and/or maternal toxicity (clinical signs or a decrease in body weight) but not death or severe suffering." While here the definition of maternal toxicity is relatively vague, the closely related and newer OECD Guideline 426 (Developmental Neurotoxicity Study, 2007) is more precise, stating that "the highest dose level should be chosen with the aim to induce some maternal toxicity (e.g., clinical signs, decreased body weight gain (not more than 10%) and/or evidence of dose-limiting toxicity in a target organ)". Based on this, a body weight gain decrease of up to 10% would be desirable, but a loss in body weight is certainly not acceptable.

To address this issue the mean net maternal body weight change, i.e. carcass weight on GD 20 minus body weight on GD 6 is considered as the most relevant parameter for maternal body weight effects being a reliable indicator of maternal toxicity during the application period (Moore et al., 2013). The carcass weight is the terminal body weight minus uterine weight. The most relevant parameters are given in the following tables in bold letters. The respective absolute and relative net body weight changes (relative to the control group) for both developmental toxicity studies are listed in the last two lines in bold letters in the tables below. For an easier comprehension of the maternal toxicity other relevant parameters like body weight on GD 20, body weight change GD 6-20, food consumption GD 6-20 and gravid uterus weight are also given.

Table 1 lists the data for the Morseth (1989) study, and table 2 those for the Pique (2014) study.

Table 1: Net maternal body weight change; Morseth (1989)

Dose level (mg/kg bw/d)	**0**	**5**	**15**	**45**
Carcass weight (g)[1)]	287.0	280.6	265.2*	243.9*
Body weight GD 6 (g)	258.8	261.7	252.8	254.3
Net absolute body weight change GD 6-20 in grams[2)] (% of control in brackets[3)])	28.2	18.9 (-33)	12.4 (-56)	-10.4 (-137)
Body weight on GD 20 (g)	364.6	355.9	334.2*	305.3*
Body weight change GD 6-20 (g)	105.8	94.2*	81.4*	51.0*
Food consumption GD 6-20 (g/day)	23.8	22.5	20.9	17.5
Gravid uterus weight (g)	77.5	75.3	69.0	61.4*

1) Carcass weight = terminal body weight minus uterine weight
2) Net body weight change = terminal body weight minus uterine weight minus GD 6 body weight
3) Relative body weight changes are calculated as not given in the original study, therefore no statistics available
GD = gestation day
* statistically significant

Table 2: Net maternal body weight change; Pique (2014)

Dose level (mg/kg bw/d)	**0**	**3**	**15**	**45**
Carcass weight (g)	315.7	315.2	300.7*	236.6*
Body weight GD 6 (g)	268.0	271.4	273.5	270.9
Net absolute body weight change GD 6-20 in grams (% of control in brackets)	47.8	43.8 (-8)	27.1* (-43)	-34.3* (-172)
Body weight on GD 20 (g)	395.9	390.7	370.5*	288.0*
Body weight change GD 6-20 (g)	133.7	125.4	102.7*	21.7*

Food consumption GD 6-20 (g/day)	27.8	27.5	25.2*	14.9*
Gravid uterus weight (g)	80.2	75.5	69.8*	51.4*

GD = gestation day
* statistically significant

Applying the criteria of the Developmental Neurotoxicity Guideline 426 (2007) for a decreased body weight gain of (not more than) 10% in the high dose group to the study of Morseth (1989) would have resulted in a net maternal body weight increase of 25.4 g and not to a loss of 10.4 g (-137%) in the high dose group.
On the other hand the net body weight gain of the mid dose animals of 12.4 g (about -56% compared to the control) comes closer to the requirement of the new OECD Guideline 426 although still exceeding the proposed limit on body weight effects of about 10 % for a high dose group.

In the recent study (Pique, 2014) the difference in net body weight gain in the high dose groups was even more pronounced: the control group dams had a net body weight gain of 47.8 g (GD 6-20) and consequently the high dose group animals should have had a net body weight gain in the range of 43 g (- 10%), but actually these animals lost 34.3 g (-172%). Even at the mid dose the net body weight gain was only 27.1 g (-43% of the control) and thus exceeding already the requirements for effects considered appropriate for a high dose group.

This difference in net body weight gain between the high dose groups of both studies may stem from differences in sensitivity of the rat strain used. However, it can as well be explained by the different dosing scheme: In the Morseth (1989) study application of HC was terminated at GD 15 so that the dams could partly recover from the toxicity of HC until sacrifice at GD 20. But within this timespan recovery by far was not complete supporting the notion of severe maternal toxicity at the high dose level. In contrast, Pique (2014) dosed until GD 19, i.e. the day before termination of the study and therefore recovery from toxicity was not possible.

In addition Pique (2014) reported that the health status of three animals of the high-dose group (3 out of 23 = 13%) was extremely poor during the first 6 days of dosing and ethical sacrifice was considered. This on its own would have constituted excessive maternal toxicity according to CLP criteria. According to UN (2013) [paragraph 3.7.2.4.4 (a)] "maternal mortality greater than 10% is considered excessive and the data for that dose level should not normally be considered for further evaluation." Consequently, due to the severity of the maternal toxicity in the high dose group, maternal and, secondary to this, fetal homeostasis were certainly compromised considerably, and therefore the high dose group should not be taken into consideration for classification purposes.

This also applies to the high dose group in the Morseth (1989) study.

In contrast, the effects noted for the mid dose groups in both studies came closer to the guideline requirements for a high dose group, although still somewhat exceeding the recommendation as described in the Developmental Neurotoxicity Guideline 426 (2007). Consequently, evaluation of the potential developmental effects of HC should be based on the findings in the mid dose group in both studies as this dose of 15 mg/kg bw/d already represents a reasonable worst case scenario with regard to the observed maternal toxicity.

<u>Conclusion, proposal for classification:</u>

The findings in the high dose groups of both studies must be seen in conjunction with the severe maternal toxicity leading to massive reductions of net body weight gain in both studies associated with an extremely compromised health status of some animals in one of these studies. Due to the severe maternal toxicity the findings observed at 45 mg/kg bw/d should not be considered for developmental toxicity classification. The observations at the mid dose level (15 mg/kg bw/d), where still clear maternal toxicity was observed would, per se not warrant a classification for developmental toxicity.
However, if the critical effect noted in the high dose groups of both studies (diaphragmatic hernias) is taken into account, the fact that for this effect the Dossier Submitter has demonstrated a specific maternally-mediated mechanism should be considered as well. Therefore, a classification into Category 2 (H361d) could be suitable for reasons of precaution.

Please find attached the following documents:
1. Developmental effects and maternal toxicity of Hydrogen cyanamide
2. Abstract of the study: Hydrogen Cyanamide – Prenatal Developmental Toxicity Study by the Oral Route (gavage) in the Rat
3. Full study report of study: Hydrogen Cyanamide – Prenatal Developmental Toxicity Study by the Oral Route (gavage) in the Rat (CONFIDENTIAL)

ECHA note: the following attachments were provided with this comment [attachments 1-3]:
1. Developmental effects and maternal toxicity of Hydrogen cyanamide
2. Abstract of the study: Hydrogen Cyanamide – Prenatal Developmental Toxicity Study by the Oral Route (gavage) in the Rat
3. Full study report of study: Hydrogen Cyanamide – Prenatal Developmental Toxicity Study by the Oral Route (gavage) in the Rat (CONFIDENTIAL)

Dossier Submitter's Response

It is noted that a new prenatal developmental toxicity study by the oral route (gavage) in the rat has been submitted (Pique, C., 2014).

In this study hydrogen cyanamide, was administered by gavage at dose levels of 3, 15 and 45 mg/kg bw/day (active ingredient) to groups of 25 mated female Sprague-Dawley rats from days 6 to 19 of gestation inclusive. Treatment-related clinical signs were restricted to the 45 mg/kg bw/day group. There was a dose-related and statistically significant reduction in overall mean body weight gain and food consumption during the dosing period (G 6 to G 20) in the 15 and 45 mg/kg bw/day groups leading to lower mean terminal body weight (-6 % and -27 % in groups 3 and 4, respectively) compared with the control. The effect in the high dose group was associated with body weight loss during the first 6 days of the dosing period (G 6 to G 12). The effect was confirmed by lower mean net body weight change (i.e. maternal body weight change from G 6 to G 20 minus gravid uterus weight) in the 15 and 45 mg/kg bw/day groups (27 g and -34 g, respectively) compared with the control (48 g). There was clear evidence of general developmental toxicity (delay in development) in the 45 mg/kg bw/day group with markedly reduced mean foetal weight (-38 %) (resulting in lower mean gravid uterus weight), widespread incomplete/absent ossification of axial and appendicular skeletal bones and thickened and/or wavy ribs (7 % and 31 %, respectively) compared with the control. There were also 7 foetuses from the same number of litters with a malpositioned (not completely descended) testis. Other malformations noted in the high dose group including 10 (7) foetuses (litters) with a small diaphragmatic hernia (no impact on the lung size) and 4 (4) foetuses (litters) with defects of the great blood vessels could not be clearly attributed to the observed maternal toxicity by the author (Pique, C., 2014).

The results of this new study confirm those of the study of Morseth (1989). In both studies

visceral and skeletal malformations associated with maternal toxicity were observed at the high dose (45 mg/kg bw/day). As discussed in detail in the CLH report (May 2014) there is evidence for a specific maternally-mediated mechanism leading to malformations for cyanamide.

According Guidance to Regulation (EC) No 1227/2008 on Classification, Labeling and Packing of substances and mixtures (3.7.2.2.1 Classification in the presence of parental toxicity; 14 May 2009 – IHCP, Dg Joint Research Centre, European Commission)
"Classification shall not automatically be discounted for substances that produce developmental toxicity only in association with maternal toxicity, even if a specific maternally-mediated mechanism has been demonstrated. In such a case, classification in Category 2 may be considered more appropriate than Category 1. …".
Since developmental effects relevant for classification (i.e. diaphragmatic hernia) were observed only in one species at a high dose level associated with significant maternal toxicity or marked systemic toxicity, classification in Category 2 (CLP) and in Category 3 (DSD) is considered appropriate.
For this reason classification concerning effects on development in Category 3 (R63, DSD criteria) and Category 2 (H361d, CLP criteria) is still considered appropriate (CLH report May 2014).

RAC's response

The RAC agrees with the DS that classification for developmental toxicity is required. However, according to the criteria, 'clear evidence of an adverse effect on development' must be demonstrated ' in the absence of other toxic effects', or the adverse effect on reproduction is considered not to be a 'secondary non-specific consequence of other toxic effects' . In the case of cyanamide, adverse developmental toxicity including specific malformation was seen in association with severe maternal toxicity in the rat. Some supporting evidence is seen in the rabbit. In addition, clear embryofoetal toxicity in conjunction with maternal toxicity was demonstrated in the multigeneration studies described above which also supports this proposal.
The mechanism proposed relates to inhibition of Raldh2 and is of relevance to humans. While general maternal toxicity was clearly present, the malformations were attributed to a separate specific mechanism. General maternal toxicity can no doubt contribute to the multiple embryofoetal effects also seen.
The RAC considers the criteria for 'clear evidence' to be fulfilled and proposes classification in Cat 1B for developmental toxicity.

Date	Country	Organisation	Type of Organisation	Comment number
07.11.2014	France		MemberState	9
Comment received				
MS FR doesn't comment on this endpoint.				
Dossier Submitter's Response				
noted				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
04.11.2014	Netherlands		MemberState	10

Comment received
The NL CA agrees with the classification of Repr. 2 (H361fd) based on effects on fertility and development. Effects on fertility: • A significant reduction in mean corpora lutea (effect on fertility), implantation sites, live rat embryos in F0 dams and testicular atrophy in F0 and F1 males at 25 mg/kg/day. The NOAEL for parental, reproductive and offspring toxicity in this gavage study was 7 mg/kg bw/day. Adverse findings on litter parameters were restricted to the F0/F1 generation and could not be reproduced in the F1/F2 generation. This study was not performed according to GLP. • 90-day gavage study in beagle dogs showed histopathological findings in testes and epididymis regarding spermatogenesis most pronounced at 6 mg/kg bw/day accompanied by reduced testes weight (p.65-74 CLH Report). • 90-day oral toxicity study in dogs showed evidence of testicular damage (p.74-80 CLH Report). • 52-week oral gavage study in beagle dogs showed histopathological changes in testis and epididymis (p. 80-88 CLH Report). Effects on development • A significant decrease in live litter size on postnatal day 4 was observed in F0 and F1 (15.41 mg/kg bw/day) in a rat two-generation study (Table 129, p. 167-168 CLH Report). There was also testicular tubular atrophy (1.66 mg/kg bw/day) and insterstitial cell proliferation (15.41 mg/kg bw/day) reported in F1 males (developmental effect as not observed in F0) (Table 132, p. 170, CLH Report). The NOAELs for parental toxicity and reproductive toxicity was 2.5 mg/kg bw/day and for developmental toxicity was 1.3 mg/kg bw/day. • A significant decrease in pregnant females and decrease in pup viability in F0 rats, and a decrease in pup viability in F1 rats in a two-generation study (Table 135, p. 177 CLP). The parental NOAEL and NOAEL for reproduction was 3.75 mg/kg bw/day, while the NOAEL for developmental toxicity was <1.25 mg/kg bw/day based on increased pup mortality. Overall, it is not directly clear whether these effects observed at dose levels also inducing maternal and general toxicity occurred secondary to maternal toxicity and general toxicity. The Netherlands agrees with no classification for lactation.
Dossier Submitter's Response
It is noted that the Netherlands agrees with the classification of Repr. 2 (H361fd) based on effects on fertility and development and agrees with no classification for lactation. See our comment to number 8.
RAC's response
The RAC agrees with comments of the NETH that classification for fertility is required. Significant adverse effects on fertility have been observed in the rat in conjunction with moderate to marked systemic toxicity, with some indications for a possible testicular effect. It is not possible to differentiate between the systemic toxicity and the observations of impaired fertility. However, notwithstanding that there are significant methodological limitations in two of these studies; the evidence for an adverse effect is clear and convincing. This is supported by evidence in a second species, an effect on the testis in three dog studies. The RAC therefore considers the evidence as sufficient for classification in Cat 1B.

RESPIRATORY SENSITISATION

Date	Country	Organisation	Type of Organisation	Comment

				number
07.11.2014	France		MemberState	11
Comment received				
MS FR doesn't comment on this endpoint.				
Dossier Submitter's Response				
noted				
RAC's response				
Noted.				

OTHER HAZARDS AND ENDPOINTS – Acute Toxicity

Date	Country	Organisation	Type of Organisation	Comment number
04.11.2014	Netherlands		MemberState	12
Comment received				
The NL CA agrees with the classification for Acute Tox. 3 (H301) and Acute Tox. 3 (H311) because of the reported oral LD50 of 142 mg/kg bw in rats (Cat. 3: Oral LD50 > 50 but ≤ 300 mg/kg bw) and dermal LD50 of 848 mg/kg bw in rabbits (Cat. 3: Dermal LD50 > 200 but ≤ 1000 mg/kg bw). The Netherlands agrees with no classification for STOT-SE.				
Dossier Submitter's Response				
It is noted that the Netherlands agrees with the classification for Acute Tox. 3 (H301) and Acute Tox. 3 (H311).				
RAC's response				
Noted.				

OTHER HAZARDS AND ENDPOINTS – Skin Hazard

Date	Country	Organisation	Type of Organisation	Comment number
04.11.2014	Netherlands		MemberState	13
Comment received				
The NL CA disagrees with the change in classification from Skin Irrit. 2 (H315) to Skin Corr. 1B (H317) because: • After one week of treatment in the skin of 6 rabbits, the greater part of the skin areas showed slight to distinct necrosis (p. 35 CLH Report). This study deviated from OECD TG404 guideline in that scoring was performed at 4 and 52 h, instead of the recommended 24, 48 and 72 h. • Supporting in vitro data (EpiDermTM (Epi-200) showed 85% viability after 3 min and 7% after 60 min (p.45 CLH Report). According to OECD TG 431, a combination of optional sub-categorization 1B/1C can be made when the viability is ≥ 50% after 3 min AND ≤ 15% after 60 min (OECD, 2014). Therefore, we disagree that sub-categorization of Skin Corr. 1B can made on the basis of in vitro results as suggested in the 'comparison with criteria in CLP regulation' (p.47, CLH Report) because with the EpiDermTM, a differentiation between 1B and 1C cannot be made (OECD 2014). The criteria for 3 minutes and 1 hour are only applicable to in vivo studies				

and not to in vitro studies. Given that the information provided is not sufficient for sub-categorization, Skin. Corr. 1 could be considered in line with the CLP Guidance chapter 3.4.2.2.4 and step 6a in the decisions logic in 3.4.2.1.6 especially as the use of Cat 1 for substances was accepted already at the GHS.

Dossier Submitter's Response

The skin irritation study of Van Beek (1982), similar to OECD TG 404, conducted with Cyanamide F1000, revealed well-defined to moderate erythema, slight ischemia, very slight incrustation and very slight edema were observed after 52 h. The treated skin areas had a purple colour suggesting the presence of haemorrhages. After one week the greater part of the treated skin areas showed slight to distinct necrosis. Hence the seen effects were not completely reversible with in the observation period. There was an exposure period of 4 h. Skin effects indicative for full thickness destruction (incrustations after 52 h and necrosis after 1 week) lead to a classification with R34 (CLH report May 2014).

After dermal exposure severe irritation can occur in humans (see section 4.12.1.6 "Human information, Cyanamide in alcohol therapy" of CLH report May 2014).

The in vitro skin corrosion test with Cyanamid L 500 using EpiDerm reconstructed skin membranes of Reus, A. A. (2011) showed 85% viability after 3 min and 7% after 60 min (CLH Report). The study of Reus, A. A. (2011) was conducted in accordance with the OECD Guideline 431 (In vitro skin corrosion: Human skin model test. Adopted 13 April 2004). According to the test the OECD Guideline 431 the test substance is considered to be corrosive to skin (CLH report May 2014).
According to the OECD Guideline 431 (Adopted 13 April 2004) the test does not normally provide adequate information on skin irritation, nor does it allow the subcategorisation of corrosive substances as permitted in the Globally Harmonised Classification System (GHS). According to the OECD Guideline 431 (Adopted 13 April 2004 or 26 September 2014) the result of the in vitro skin corrosion of Reus, A.A. (2011) allows to constitute a combination of optional sub-categories 1B and 1C.

Therefore, we agree to Netherlands that sub-categorization of Skin Corr. 1B cannot be made on the basis of in vitro results as suggested in the 'comparison with criteria in CLP regulation' (CLH Report May 2014) because with the EpiDermTM, a differentiation between 1B and 1C cannot be made (OECD Guideline 431 Adopted 13 April 2004 or 26 September 2014). The criteria for 3 minutes and 1 hour are only applicable to in vivo studies and not to in vitro studies. Thus given that the information provided is not sufficient for sub-categorization, Skin. Corr. 1 with the hazard statement H314 (Causes severe skin burns and eye damage) could be considered in line with the CLP Guidance.

The skin effects (necrosis) in the in vivo study with Cyanamide F1000 persisted until the end of the observation period. There was an exposure period of 4 h leading to a classification with skin corrosion category 1 C (H314).
Overall, the classification into category 1 is required. The results of the in vitro study are preferred compared to the results of the in vivo study, based on the better study conduct (i.e., limited times of scoring of the animal's skin reactions), hence a classification into category 1 with the hazard statement H314 (Causes severe skin burns and eye damage) is proposed.

RAC's response

Note: Can the DS confirm that van Beek 1982, conducted with Cyanamid F1000, referred to here is different to van Beek 1982, conducted with Cyanamid L500, which was submitted for the PPP evaluation. The test house report number (B82-0061-4 p. 46 CLH report) is the same for both.

RAC agrees with the Neth/DS that the results of the van Beek, 1982, support classification as corrosive irrespective of the non-guideline observation time of 52 hours as necrosis was visible at study termination, day 7.

OTHER HAZARDS AND ENDPOINTS – Eye Hazard

Date	Country	Organisation	Type of Organisation	Comment number
04.11.2014	Netherlands		MemberState	14
Comment received				
The NL CA agrees with removal of Eye Irrit. 2 (H319) because cyanamide is classified as skin corrosive and the risk for severe eye damage is implicit (CLP Annex I 3.3.2.3). However, as explained in the CLP guidance (3.3.2.4) this means that the substance requires both classifications (Skin Corr. 1 and Eye Dam. 1) but no labelling with H318 (CLP article 27). Therefore, Eye Dam. 1 (H318) should be considered.				
Dossier Submitter's Response				
According to CLP guidance (3.3.2.4.) a skin corrosive substance is considered to also cause serious eye damage which is indicated in the hazard statement for skin corrosion (H 314: Causes severe skin burns and eye damage). Thus, in this case both classifications (Skin Corr. 1 and Eye Dam. 1) are required but the hazard statement H318 'Causes serious eye damage' is not indicated on the label because of redundancy (CLP Article 27). The results of the studies of Ligget, 1991 and van Beek, 1974 meet the DSD (Xi, R36) and CLP criteria (eye irritation cat. 2, H319). However as the substance is skin corrosive (cat. 1B, H314 according to CLP criteria and C, R 34 according to DSD criteria), specific labelling as an eye irritant is not necessary, because it is already included implicitly in the classification as skin corrosive. In agreement with Netherlands this means that the substance requires both classifications (Skin Corr. 1 and Eye Dam. 1) but no labelling with Eye Dam. 1 (H318) should be considered.				
RAC's response				
The RAC agrees with the proposal to classify as H314 (Skin Corr. 1 and Eye Dam. 1) and not to include H318 in the label as indicated by CLP Art 27. The NL comment seems to indicate that H318 should be considered. RAC does not agree with the NL CA that H318 should be considered for this reason (CLP Art 27).				

OTHER HAZARDS AND ENDPOINTS – Skin Sensitisation Hazard

Date	Country	Organisation	Type of Organisation	Comment number
04.11.2014	Netherlands		MemberState	15
Comment received				
The NL CA agrees with the proposed Skin Sens. 1B (H317). <u>Buehler assay</u> o Criteria for Cat. 1B classification in the Buehler assay: ≥ 15% incidence of sensitized Guinea pigs with > 20% intradermal induction (Table 3.4.2-h CLP) o Results: a 40% dermal induction resulted in a 20% incidence of sensitized Guinea pigs (Table 41, p. 53 CLH Report). o The BAuA concluded that these results were inconclusive (Section 4.6.1.5; p. 53, CLH Report) o Conclusion: these results meet criteria for Cat. 1B according to CLP due to low incidence of sensitisation.				

Guinea pig maximization test o Criteria for Cat. 1B classification in the Guinea pig maximization test: ≥ 30% incidence of sensitized Guinea pigs with > 1% intradermal induction (Table 3.4.2-g CLP) o Results: a high intradermal induction dose was used (5%) where 100% of the Guinea pigs were sensitized (Table 41, p. 53 CLH Report) o Conclusion: these results meet the criteria for Cat. 1B but because a high intradermal induction dose was used, the possibility of the cyanamide being a Cat. 1A (H317) cannot be excluded. Further clarification on sub-categorisation to Skin Sens. 1B is recommended.
Dossier Submitter's Response
According to the classification by Magnusson and Kligman, cyanamide had skin-sensitising properties: after dermal application of SKW Cyanamide F 1000 (pure active substance) all Albino guinea pigs showed a positive response in the challenge test. According to the method of Buehler an aqueous solution of cyanamide (53 % w/v) induced some positive skin reactions after the challenge application (4/20). Indications for a severe irritating effect of cyanamide was noticed in one of these rabbits due to histological examination, whereas no histological findings showed any sign of delayed hypersensitivity. Thus, the results of the Buehler test were inconclusive. In contrast, the more sensitive M&K test clearly demonstrated a potential for skin sensitisation. Based on the results in the study according to M&K design, cyanamide fulfils the criteria in DSD to be classified as a skin sensitiser (R43). Based on the results in the study according to M&K design, cyanamide also fulfils the criteria in CLP regulation to be classified as a skin sensitiser (H317, sub-category 1B) (CLH report May 2014).
RAC's response
The RAC agrees with the NL CA and the DS that the M&K assay meets the criteria for Skin sensitisation Cat 1B; H317. However, as indicated by the NL CA, sub-categorisation is not considered appropriate as the induction dose was too high to differentiate between Category 1B and 1A. Classification as Category 1; H334 is hence recommended.

OTHER HAZARDS AND ENDPOINTS – Specific Target Organ Toxicity Repeated Exposure

Date	Country	Organisation	Type of Organisation	Comment number
04.11.2014	Netherlands		MemberState	16
Comment received				
The NL CA disagrees with the classification of STOT-RE 1 (H410, thyroid) because: • There were multiple effects in the 28-day rat study including: o Histopathological lesions in the thyroid, spleen and liver (Table 47, p. 58, CLH Report) o reduced body weight (Table 42,p. 42, CLH Report), reduced body weight gain (Table 43, p. 55, CLH Report) and anemia (Table 44, p. 56, CLH Report). • In the 90-day rat study, thyroid effects (small follicular lumens without colloid, separated by proliferating epithelial cells and interfollicular cells) were clearly seen in the high dose group (Table 52, p. 61, CLH Report). This study was performed at such low dose levels (up to 4.5 mg/kg bw/day) that more severe effects cannot be excluded. • Clear decreases in T3 and T4 were observed in the 90-day dog study (Table 63, p. 70, CLH Report). • In the 1-year dog study, anemia and changes in the thyroid were reported (decrease in T4; Table 77, p. 83 CLH Report). These studies were performed at really low doses (up to 6 mg/kg bw/day).				

• Toxicity is supported by the results of the chronic toxicity study in which after 16 weeks the dose was reduced from 30 mg/kg bw/day to 7.5 mg/kg bw/day because of general health concerns.

Overall, a clear functional thyroid impairement was not demonstrated and the dose levels in the 90-day and 1-year dog studies were clearly below the upper limit for STOT RE 2. These data therefore do not support a STOT-RE 1 (H410, thyroid) classification but rather a STOT RE 2 (H411) classfication. The rationale for selecting such low doses for the repeated dose toxicity studies should be included.

References:
EFSA (2010) http://www.efsa.europa.eu/en/efsajournal/doc/1873.pdf

OECD (2014) OECD TG 431: In vitro skin corrosion: reconstructed human epidermis (RHE) test method. http://www.oecd.org/chemicalsafety/testing/Draft-TG431-skin-corrosion-july14.pdf

Dossier Submitter's Response

According to the Guidance on the Application of the CLP Criteria substances are classified as specific target organ toxicants following repeated exposure by the use of expert judgement, on the basis of the weight of all evidence available, including the use of recommended guidance values which take into account the duration of exposure and the dose / concentration which produced the effect(s), and are placed in one of two categories, depending upon the nature and severity of the effect(s) observed. Classification to category 1 can be based on observations from appropriate studies in experimental animals in which significant and/or severe toxic effects, of relevance to human health, were produced. The standard animal studies in rats or mice that provide this information are 28 day, 90 day or lifetime studies (up to 2 years) that include haematological, clinicochemical and detailed macroscopic and microscopic examination to enable the toxic effects on target tissues / organs to be identified (Guidance on the Application of the CLP Criteria (Version 4.0 – November 2013)).
In short-term oral toxicity studies, significant toxic effects in the thyroid were observed in males and females of two strains of rats. The morphological changes in the thyroid in both studies were comparable. They have toxicological significance and are considered to be of relevance to human health. The lowest effect dose was seen at 4.5 mg/kg bw/day in a 90-day dietary study (Til et al., 1975). At 4.5 mg/kg bw/day in the thyroid predominantly small follicular lumens without colloid, separated by proliferating epithelial cells and interfollicular cells were seen in males as well in females (Til et al., 1975), comparable to the effects found in the 4-week study. In a 28-day gavage study the lowest effect dose was at 10 mg/kg bw/day in male rats and at 20 mg/kg bw/day in female rats (Osheroff, 1988). There was an increased incidence of follicular cell hyperplasia at 10 mg/kg bw/day and higher in male rats. Decreased colloid content and small and closely packed follicles were seen already at 5 mg/kg bw/day in the males, albeit to a marginal extent. The females were less sensitive: thyroid effects were seen dose-dependently at 20 and 40 mg/kg bw/day. At 40 mg/kg bw/day, the mean TSH values were increased by 100 %, whereas T4 concentrations were decreased by 28 % in both sexes compared to control animals (Osheroff, 1988).
Thus, it can be concluded that classification for this endpoint is required because the effect levels were below the threshold dose levels set in DSD and CLP regulation. According to CLP criteria, category 1 is proposed (CLH report May 2014).

RAC's response

The RAC agrees with the argument presented by the DS. In addition, the finding in a second species of hypothyroidism at dose levels within the criteria for Category 1 supports this proposal. A case for Category 2 could be supported by the known relative sensitivity of rats to antithyroid effects through interference with the thyroid-pituitary axis. However, the data

from the 90-day and 1-year studies indicate significant sensitivity of the dog also. The basis for assuming that dogs and rats are equally more sensitive than humans is somewhat uncertain in the case of the dog.
The conclusion of the NL comment is not clear. The reasoning why the data should support Category 2 and not Category 1 seems somewhat contradictory; all effects in both rats and dogs are within the numerical range of the Category 1 criteria. In addition, significant alteration in T4 and T3 would indicate clear functional disorder, therefore supporting that this is a 'significant' effect.

Date	Country	Organisation	Type of Organisation	Comment number
10.11.2014	Germany	AlzChem AG	Company-Manufacturer	17
Comment received				

Effects of cyanamide (CY) on the thyroid in relation to the proposed classification STOT RE1

Summary:
CY acts on the thyroid gland by inhibition of thyroid peroxidase (TPO) that may lead to a decrease of T3 and T4 in blood accompanied by an increase of TSH followed by stimulation of thyroid activity. Including TPO inhibition there are 5 different mechanisms of action (MOA) associated with the same sequence of effects on thyroid homeostasis. However, in all cases rats are by far more susceptible than humans and the sensitivity of rats and dogs is assumed to be comparable. The high sensitivity of rats and dogs in comparison to primates has specifically been demonstrated for sulfonamides, an important group of TPO inhibitors, and therefore the same species difference in sensitivity can also be inferred for CY. The high sensitivity of rats and dogs stems from their lack or limited amounts of the high affinity thyroxine binding globulin (TBG) the major transporter of T4 in primates acting as a "buffer" against fluctuations of thyroid hormones.

CY has been extensively used in the treatment of alcoholism and in more than 700.000 patients receiving average daily doses of 1 mg/kg bw/d over up to several years. No indications for clinically relevant disturbances of the thyroid became apparent. This NOAEL of 1 mg/kg bw/d in humans can be metabolically scaled to 4 mg/kg bw/d for rats, which is by a factor of 4 higher than the NOAEL obtained in the chronic study of the highly susceptible rat. This supports that the rat is not a suitable model to quantitatively predict thyroid effects in humans. On one hand for STOT classification reliable and relevant human data are preferable, on the other hand the trigger values for STOT 1 and 2 are referring to doses and effects noted in 90 day rat studies. Therefore this NOAEL, obtained by using a scaling factor of 4 between rats and humans, is used as more reliable starting point since rats and dogs are far more sensitive than humans. In addition if animal data are used for STOT classification they should show severe or significant toxic effects in the target organ in the dose ranges given and should be of relevance for humans. Although TPO inhibition is of relevance for humans, the dose levels causing effects in animal experiments are clearly misleading for an extrapolation to humans. From the experimental findings with CY, a scientifically justified, objective gradation of severity or significance of toxicity is very difficult and can be controversial. But the most severe consequence of chemicals affecting thyroid homeostasis, namely tumour induction, has not been observed in carcinogenicity studies with CY at the same dose levels as those used in 90 day and 2 year repeated dose studies. In addition prolongation of exposure did not lead to a decrease of the NOAELs for rats and dogs.

For STOT classification, the broad database in humans and the high sensitivity of rats and dogs in comparison to humans should be taken into account. The epidemiological NOAEL in humans should be compared with the NOAEL in the chronic rat study using the metabolic

scaling factor of 4 to extrapolate from humans to rats. Thereby it can be shown that humans are at least by a factor of 4 less sensitive than rats for effects on the thyroid gland. This difference in sensitivity must be taken into due account for extrapolating the experimental LOAELs to humans. It is shown that such extrapolated LOAELs are above the classification limit for STOT RE1 regardless of whether the effects are considered as "severe" or "significant". Therefore STOT RE1 is not justified and STOT RE2 would be appropriate.

A more detailed description on our STOT RE classification proposal is available in the attached document "cyanamide STOT RE classification.pdf".

ECHA note: The following attachment was submitted with the above comment [Attachment 6]:
Effects of cyanamide (CY) on the thyroid in relation to the proposed classification STOT RE1

Dossier Submitter's Response

The proposal of AlzChem AG to apply a scaling factor of 4 between rats and humans is registered.
See our comments to number 2 and 16

RAC's response

The known sensitivity of the rat to substances interfering with circulating thyroid hormones is recognised. That this also applies to the dog is less convincing and not clearly supported by the published literature.
It is acknowledged that Colme (hydrogen Cyanamid) has been used for in excess of 2 million alcoholic patients apparently without significant adverse effects. It is noted that the information relating to the occurrence of thyroidism in patients only appears in Comment 2 above from industry and is not mentioned in the CLH report. According to the industry, hypothyroidism in hydrogen cyanamide treated patients is considered rare (>1/10000; <1/1000),i.e. it occurs in humans, albeit at a low frequency.
They also remark that during the 18-year period from 1996 to 2014 no case of adverse effects on the thyroid has been reported to the company nor has any case of adverse thyroid effects been reported in the more recent literature.
This indicates that there is some, but very weak support from the human evidence, for an anti-thyroid effect in humans. It is also noted that the population of alcoholic patients should possibly not be considered as representative of the normal population, due the possibility of liver disease or other unknown confounding factors.

OTHER HAZARDS AND ENDPOINTS – Hazardous to the Aquatic Environment

Date	Country	Organisation	Type of Organisation	Comment number
07.11.2014	France		MemberState	18

Comment received

Please find below minor comments:

- 2.2 Short summary of the scientific justification for the CLH proposal.
The new proposal for classification for environment is not reported in this section and could be added.

- 5.3.1.1 Bioaccumulation estimation.
The surface tension value (65.2 mN/m) is different that the surface tension reported in the Table 10 (72.86 mN/m). Could you please check?

Dossier Submitter's Response
To 2.2: Agree. To 5.3.1.1: Thank you, the correct value is 72.86 mN/m.
RAC's response
Noted.

Date	Country	Organisation	Type of Organisation	Comment number
10.11.2014	United Kingdom		MemberState	19
Comment received				
Whilst we note that the CLH report can not be amended at this stage, we do not feel the current proposal includes sufficient detail on fate or ecotoxicity information to agree the environmental classification. We have the following specific comments. Fate: In our opinion, the aquatic simulation study (Völkl, 2000) included in the DAR and CLH IUCLID file should have be presented and discussed in the CLH report. This study quotes whole system DT50 values of 2.5-4.8 days with >80% mineralisation by day 28 and identification of the principle degradant (urea). On this basis, the study should be included when considering if the substance is rapidly degradable or not rapidly degradable and the relevance of degradants. Ecotoxicity: The CLH report states that the A. Flos-aquae study (Hertl, 2000) is not valid. However, neither the CLH report nor the IUCLID clearly present information (such as discussion of control data) to support this and the study is given a reliability rating of 1 in the IUCLID. On this basis, the rationale and data to exclude the study endpoints should be explained as algae are a key species and inclusion/exclusion determines whether the substance should be classified for acute effects or not.				
Dossier Submitter's Response				
Ecotoxicity: This growth inhibition test with *Anabaena flos-aquae* is not valid because of the following reason: Growth rate is the preferred evaluation parameter from an algae growth inhibition test. To enable a proper growth rate evaluation the control culture must show exponential growth over the whole exposure period. However, the control replicates of this test do not grow exponentially: According to OECD guideline 201 the specific growth rate for *Anabaena flos-aquae* should be between 1.1 – 1.4 per day. In this test the daily growth rates are 2.6 between day 0 and 1, 0.33 between day 1-2 and 0.44 between day 2-3. The mean coefficient of variation for section-by-section specific growth rate is 114%. Consequently this test cannot be used for the effect assessment for cyanamide and a reliability of 3 is appropriate to this study. If there is a reliability rating of 1 given in the IUCLID then it is a typing error. This study was given a reliability factor of 3 in the frame of the approval of cyanamide as an existing biocidal active substance. As there is another valid algal growth inhibition test available (*Pseudokirchneriella subcapitata*) for cyanamide, a repeat of the study was not required. Fate: Thank you for the comment. We did not consider the water-sediment study as we interprete point b and c as referring to a water simulation study (OECD 309) which is not available. Our understanding of the decision scheme is that a water-sediment simulation				

study (point d) is only considered if none of the studies mentioned under point a-c is available. For this reason we used the ready biodegradability study for the decision only. However, as in the water-sediment study nearly most of the radioactivity was found in the water phase in a weight of evidence consideration of the water-sediment study is possible and cyanamide can be considered as rapidly degradable. As the observed metabolites except urea are below 10% they need not to be considered. For the major metabolite urea (max. 13.4 % in pond system) DT50 values of 5.5 days (river) and 15.2 days (pond) were determined. Thus, urea can also be considered as rapidly degradable.

RAC's response

Ecotoxicity: RAC accepts the explanation by the DS and considers the Hertl (2000) study invalid. Details are presented in the opinion.
Fate: RAC accepts that the primary study for determination of rapid degradability in this case is the ready biodegradability study. However, this does not preclude the use of data in alternative studies if they already exist and so this can facilitate a weight-of-evidence approach. As correctly pointed out by the UK MS, this study should have been accessed and reported in the CLH report. Indeed, industry correctly quotes in their submission document "Comment on Section 5 of the CLH Report for Cyanamide: Environmental Hazard Assessment" Annex I §4.1.2.9.2 of the CLP Regulation (EC) No 1272/2008. This states explicitly that even with a fail in the screening test other evidence may be considered and indicate potential rapid degradation in the environment. In this case the ready biodegradability study indicates no biodegradability but the water-sediment study indicates quite the opposite. RAC considers cyanamide as readily degradable in the environment.

Date	Country	Organisation	Type of Organisation	Comment number
06.11.2014	Germany	AlzChem AG	Company-Manufacturer	20

Comment received

Environmental hazard classification:

The proposal Aquatic Chronic 1; H410, Chronic M-factor 1 is not agreed with considering the degradability and the aquatic toxicity level of cyanamide.

Degradability:
• According to the CLP regulation (EC) 1272/2008 (Annex I 4.1.2.9.2) proof of rapid degradation of a substance under environmentally realistic conditions is considered relevant for classification.
• Cyanamide is subject to photodegradation in aqueous media. When exposed to light cyanamide degrades about four times faster than in the dark.
• A new study not considered in the CLH report shows that cyanamide is photodegraded in soil as under irradiation cyanamide degrades about three times faster than in the dark.
• Cyanamide is readily biodegradable in a modified OECD 301 B test system. With sodium acetate as additional substrate cyanamide degraded completely within 13 days.
• The rapid degradation of cyanamide under environmentally realistic conditions is confirmed by an aerobic water/ sediment study (aquatic systems: river and pond) not considered in the CLH report. In both systems cyanamide was degraded > 80 % within 28 days (trigger value > 70 %).

Aquatic toxicity:
• Whereas we agree with the dossier submitter that Daphnia magna is the most sensitive aquatic species we identified several flaws in the respective study report (Murrell et al., 1995) and in the CLH report which needed to be corrected. Taking these issues into

consideration the corrected 21-day NOEC for pure cyanamide is 0.1044 mg/l.

Conclusion:
As cyanamide is rapidly degraded under environmentally realistic conditions and the trigger value for the most sensitive species (0.1 mg/l) is exceeded cyanamide is to be classified as Aquatic Chronic 3; H412 "Harmful to aquatic life with long lasting effects".

Please note that we could not upload the unpublished and confidential studies not cited in the CLH report. These studies (Burri, R. (2001), Schmid, J. (1990) and Völkl, S. (2000) have been submitted to the RMS Germany and were assessed in the draftCAR under BPD which was distributed by the EU commission on 1st August 2013. Therefore, theses studies can be obtained from the competent authority Germany.

ECHA note: The following attachments were submitted with this comment [Attachment 4 and 5]:

- Comment on Section 5 of the CLH Report for Cyanamide: Environmental Hazard Assessment
- Clarification on the identity of the test substance and on resulting NOEC. "Murrell, H.R. and Leak, T. (1995): Chronic Toxicity of Hydrogen Cyanamide to Daphnia magna under Flow-Through Test Conditions; ABC Laboratories, Columbia (Missouri), Final Report #41942".

Dossier Submitter's Response

Aquatic toxicity: Thank you for clarification on the identity of the test substance which is used in the reproduction test with *Daphnia magna*. We agree with the 21-day NOEC for pure cyanamide of 0.1044 mg/L. The classification of cyanamide should be amended accordingly by RAC.

Degradability: Thank you very much for your comments. We did not consider the water-sediment study as we interprete point b and c as referring to a water simulation study (OECD 309) which is not available. Our understanding of the decision scheme is that a water-sediment simulation study (point d) is only considered if non of the studies mentioned under point a-c is available. For this reason we used the ready biodegradability study for the decision only. However, as in the water-sediment study nearly most of the radioactivity was found in the water phase in a weight of evidence consideration of the water-sediment study is possible and cyanamide can be considered as rapidly degradable. As the observed metabolites except urea are below 10% they need not to be considered. For the major metabolite urea (max. 13.4 % in pond system) DT50 values of 5.5 days (river) and 15.2 days (pond) were determined. Thus, urea can also be considered as rapidly degradable.
Regarding the OECD 301 B test: this test is only supplementary information due to considerable deviations from OECD guideline 301 concerning the concentration of cyanamide applied. However, the results of this study confirm the characterisation of cyanamide in the key study as not readily biodegradable. In an additional, modified investigation in this OECD 301 B study addressing the biodegradability of cyanamide when it serves as the only nitrogen source, degradation rates up to 100% at day 13 were obtained. However, this result is not relevant for the assessment of ready biodegradation of cyanamide due to considerable deviations from the OECD 301 guidelines. We think that it is not necessary to include this test in the CLH report.
Regarding photodegradation, we think that the relevant studies are already included in the CLH report.

RAC's response

Noted. The clarifications provided by industry support (1) revised NOEC and LOEC

endpoints, relative to the test doses used, (2) confirm the actual test concentrations of pure cyanamide used in the Murrell & Leak, (1995) study, (3) provide a revised NOEC value of 0.1044 mg a.i./L and (4) support classification with Aquatic Chronic 3 – H412 "Harmful to aquatic life with long lasting effects".

Date	Country	Organisation	Type of Organisation	Comment number
06.11.2014	Belgium		MemberState	21

Comment received

Based on the results of the aquatic toxicity test on the most sensitive species (Daphnia magna with 72hEC50=3.2mg/l, 21dNOEC=0.05mg/l, the fact that the substance is not rapidly degradable it is justified to classify, following the classification criteria of the regulation 1272/2008, as Aquatic Chronic 1, H410. Furthermore, the substance shows no potential to bioaccumulate.

In view of the proposed classification, non-rapid degradability and toxicity band for chronic toxicity between 0.01mg/l and 0.1 mg/l, an M-factor for chronic toxicity of 1 should be assigned.

In conclusion: we agree with the proposed environmental classification by BAuA.

Some editorial or/and minor comments:
Not all aquatic toxicity data are reported in the CLH report, more studies can be found i.e. in the REACH registration dossier and the RA under the pesticides regulation. The CLH report should be a stand-alone document containing ALL available data (whether reliable or not) in order to make an independent and correct evaluation on the classification of the substance and to determine the M-factors.

Dossier Submitter's Response

Thank you for your comment. However, the proposed classification has changed, please see the comments above.

RAC's response

Further clarification of the data in the CLH report and of the data originally presented in the *Murrell & Leak, (1995)* report was submitted by industry in a position paper on the Daphnia magna chronic toxicity study. The detailed analysis of the various relevant endpoints in the CLH report and unambiguous identification of the substance enable a revision of the 21-d NOEC for pure cyanamide to 0.1044mg/L and a reassessment of cyanamide as readily degradable under realistic environmental conditions. This impacts on classification because the trigger value for the most sensitive species (0.1 mg/L) is exceeded, an amended classification of Aquatic Chronic 3; H412 "Harmful to aquatic life with long lasting effects" is supported by RAC.

ATTACHMENTS RECEIVED

1. Developmental effects and maternal toxicity of Hydrogen cyanamide. Submitted by AlzChem AG on 05.11.2014 (Filename: Dev effects and maternal tox.pdf) *[Please refer to Comment 8]*

2. Abstract of the study: Hydrogen Cyanamide – Prenatal Developmental Toxicity Study by the Oral Route (gavage) in the Rat. Submitted by AlzChem AG on 05.11.2014. (Filename: Abstract prenatal dev tox study.pdf) *[Please refer to Comment 8]*

3. Full study report of study: Hydrogen Cyanamide – Prenatal Developmental Toxicity Study by the Oral Route (gavage) in the Rat (CONFIDENTIAL). Submitted by AlzChem AG on 05.11.2014. (Filename: prenatal dev tox study_2014.pdf) *[Please refer to Comment 8]*

4. Comment on Section 5 of the CLH Report for Cyanamide: Environmental Hazard Assessment. Submitted by AlzChem AG on 06.11.2014 (Filename: detailed environ hazard comments.pdf) *[Please refer to Comment 20]*

5. Clarification on the identity of the test substance and on resulting NOEC. "Murrell, H.R. and Leak, T. (1995): Chronic Toxicity of Hydrogen Cyanamide to Daphnia magna under Flow-Through Test Conditions; ABC Laboratories, Columbia (Missouri), Final Report #41942". Submitted by AlzChem AG on 06.11.2014 (Filename: Position paper_Daphnia magna chronic toxicity.pdf) *[Please refer to Comment 20]*

6. Effects of cyanamide (CY) on the thyroid in relation to the proposed classification STOT RE1 Submitted by AlzChem AG on 06.11.2014 (Filename: cyanamide STOT RE classification.pdf) *[Please refer to Comment 17]*