Comment on Section 5 of the CLH Report for Cyanamide:
Environmental Hazard Assessment

5.1 Degradation

5.1.1 Stability

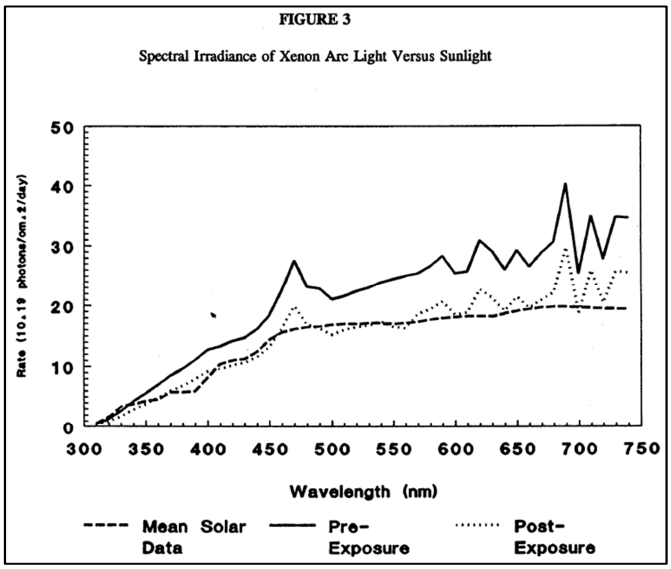
5.1.1.1 Hydrolytic degradation of cyanamide

No comment on this section.

5.1.1.2.1 Photolysis in water

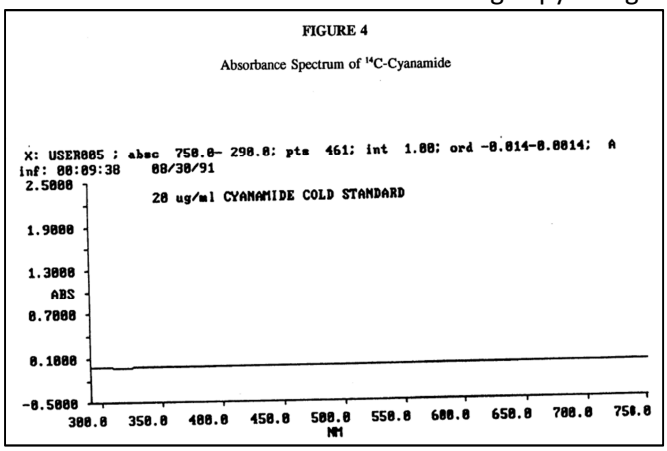
Subject: According to the CLH Report "*it can be concluded that in contrary to the laboratory conditions natural irradiation in Central Europe does not cause relevant direct photolytic degradation*". ***In our opinion this statement is not justified.***

Explanation: According to the mentioned reference (Schmidt, JM (1991), Doc No 712-001, Doc IIIA, Section A7.1.1.1.2/01) the spectral irradiance of Xenon arc light was very similar to the spectral irradiance of sunlight in a wavelength range from 290 nm to 750 nm as it can be seen in the following copy of Figure 3 of the reference:



Quantum yield was not determined in the study since the absorption coefficient at 290 nm was below 10 in UV-VIS spectra. The CLH report concludes that therefore "*in contrary to the laboratory conditions natural irradiation in Central Europe does not cause relevant direct photolytic degradation*".

The absorbance spectrum of cyanamide was constant and below 10 for all wavelengths tested, ranging from 290 nm to 750 nm as it can be seen in the following copy of Figure 4 of the reference:



Nevertheless, light exposed samples of cyanamide degraded about four times faster than dark control samples (Table 155, CLH Report). It was therefore correctly concluded in the study report that "*the results of this study demonstrate that* ***cyanamide is subject to photodegradation in aqueous media***".

5.1.1.2.2 Phototransformation in air

Subject: According to the CLH Report "Cyanamide is stable in the atmosphere" according to the Atkinson calculation. It is however mentioned in the report that this calculation is questionable because cyanamide is not a molecule that is well suited for this model. We do agree that this calculation is not well suited for cyanamide and we would furthermore like to add our thoughts to the possible appearance of cyanamide in the atmosphere.
Explanation: Its vapour pressure of 0.51 Pa at 20 °C is an indicator of a possible volatilisation potential of cyanamide. However, the Henry's law constant calculated as 2.68×10^{-5} Pa m^3 mol^{-1} indicates that the high water solubility of cyanamide is likely to counteract the tendency of cyanamide to volatise from water and moist soil surfaces.
Furthermore, cyanamide is rapidly degraded in the environment. A DT_{50}field value of 1.26 days was determined in a study on aerobic soil metabolism (Schmidt, J., 1990) and of 4.3 days in surface water (please refer to chapter 5.1.2.1.3). The photolytic half-life of cyanamide on soil surfaces is even shorter with 1.45 days (please refer to chapter 5.1.1.2.3). Therefore, it can be assumed that the fast degradation of cyanamide in soil and surface water is likely to limit volatilisation after application. These summarized findings indicate that independent of the likelihood of phototransformation of Cyanamide in the air, it is unlikely that relevant concentrations of Cyanamide do appear in the atmosphere.

5.1.1.2.3 Phototransformation in soil
Subject: Studies have been performed on the phototransformation potential of Cyanamide in soil. But these studies are neither presented nor considered for the overall assessment of the stability of Cyanamide in the CLH Report.
Explanation: In the following a summary of the key study on phototransformation potential of cyanamide in soil is presented. Reference (Burri, R. (2001), Doc No 724-002): "*Photolysis of ^{14}C-Cyanamide on soil surface under laboratory conditions*".

Method/ Guideline	Initial test substance concentra-tion	Total recovery of test substance [% of appl. a.s.]	Photolysis rate	Degradation products [% of applied radioactivity]	Half-life ($t_{1/2E}$) (days)
Directive 95/36/EC of July 14, 1995, §7.1.1.1.2 "Soil photolysis" under consideration of EPA Pesticide Assessment Guidelines, Subdivision N, Section 161-3, Photodegrada-tion studies on soil, 1982	7.5 mg a.s. per 25 cm^2 of clay loam soil plates (corr. to the max. recom-mended field rate of 30 kg a.s. per ha)	Mass balance: 93.8 ± 6.1% **(light exposed)** (mean)	**Exposed:** *Extractable radioactivity:* 102.7% (day 0) 29.6% (day 30) *Non-extractable radioactivity:* 0.3% (day 0) 12.8% (day 30) *Evolved as $^{14}CO_2$:* 41.0% (day 30)	Dicyandiamide Day 16 (max): 10.3% Day 30: 6.9% Urea: Day 6 (max): 48.4% Day 30: 18.7% Deg. product 3: unknown Max. 4.0% Deg. product 4: unknown Only on day 30: 1.4%	**Exposed:** 1.45 days

Method/ Guideline	Initial test substance concentra-tion	Total recovery of test substance [% of appl. a.s.]	Photolysis rate	Degradation products [% of applied radioactivity]	Half-life ($t_{1/2E}$) (days)
		97.9 ± 4.3% **(dark control)** (mean)	**Non exposed:** *Extractable radioactivity:* 102.7% (day 0) 8.7% (day 30) *Non-extractable radioactivity:* 0.3% (day 0) 12.7% (day 30) *Evolved as $^{14}CO_2$:* 70.6% (day 30)	Dicyandiamide Day 12 (max): 8.7% Urea: Day 12 (max): 18.4% Deg. product 3: unknown Day 6 (max): 3.4% Deg. product 4: unknown Only on days 16 and 30: 0.9-1.0%	**Non exposed:** 4.22 days

a.s. = pure cyanamide

The following conclusion is drawn in the study report:
"***In conclusion, cyanamide was rapidly degraded in the irradiated samples with a photolytical half-life of 1.45 days.*** *The main degradation product detected in the irradiated samples was urea which was then further degraded to CO_2. A degradation of cyanamide could also be observed in the dark control samples with a calculated DT_{50} value of 4.22 days. The metabolite patterns in the irradiated and non-irradiated soil samples were very similar indicating that the same degradation mechanisms were involved and that besides photolytic degradation also biotic and abiotic degradation of cyanamide in the dark plays an important role in the disappearance of cyanamide from soils.*"

5.1.2 Biodegradation
5.1.2.1 Screening tests
5.1.2.1.1 Ready Biodegradability according to OECD 301E

Subject: The concentration of the active ingredient used in the test according to OECD 301E has to be corrected in the CLH report by division by a factor of 2.
Explanation: Throughout several tests that have been performed with the test substance cyanamide there appears to be confusion with the actual concentration of "active substance" used in the test. This confusion results from the fact that some tests have been performed with pure hydrogen cyanamide whereas other tests have been performed with a 50 % aqueous solution of hydrogen cyanamide.
In the CLH report the test on ready biodegradability according to OECD 301E is mentioned. The test substance concentration has to be corrected as follows, even though this correction doesn't change the test result:

Table 157: Ready biodegradability

Method/ Guideline	Test type	Test parameter	Inoculum			Additional substrate	Test substance Concentration	Degradation		Reference
			Type	Concentration	Adaptation			Incubation period	Degree [%]	
OECD 301 E	ready	Dissolved organic carbon	Activated sludge	2 mg suspended solids/l	No	No	29.4 and 58.8 mg a.s./l correction: 14.7 and 29.4 mg pure cyanamide per litre	28 d	0	van der Hoek, E; Hanstveit, AO (1988), Doc No 713-001; Doc IIIA, 7.1.1.2.1/01

The following copy of the study report explains this in detail:

SUMMARY AND CONCLUSIONS

The biodegradability of the product Aqueous Hydrogen Cyanamide was determined by the method laid down in the OECD Guideline 301 E; 'Ready Biodegradability: Modified OECD Screening Test'(1).

Aqueous Hydrogen Cyanamide solution was tested in two concentrations: 30 $mg.l^{-1}$ and 60 $mg.l^{-1}$, corresponding to 14.7 and 29.4 mg active ingredient per litre.

The control test showed that the activity of the inoculum was sufficient, and that there was no inhibition of the inoculum activity by Aqueous Hydrogen Cyanamide. The test substance was stable under sterile conditions. No biodegradability was found in the test bottles; Aqueous Hydrogen Cyanamide is not regarded as readily biodegradable.

5.1.2.1.2 Ready Biodegradability according to a test similar to OECD 301B

Subject1: As explained in chapter 5.1.2.1.1 the concentration of the active ingredient used in the test according to OECD 301B also has to be corrected in the CLH report.
Subject 2: The study was performed with and without the use of an additional substrate, which is not mentioned in the CLH report. The additional substrate considerably changes the outcome of the test.
Explanations: In the CLH report also the test on ready biodegradability similar to OECD 301B is mentioned. The test substance concentration has to be corrected here as shown in the following modified copy of the CLH Report. Additionally degradation of cyanamide in the presence and absence of 100 mg /l sodium acetate should be compared when interpreting the test results.

Table 157: Ready biodegradability

Method/ Guideline	Test type	Test parameter	Inoculum			Additional substrate	Test substance Concentr	Degradation		Reference
			Type	Concentration	Adaptation			Incubation	Degree [%]	
OECD 301 E	ready	Dissolved organic carbon	Activated sludge	2 mg suspended solids/l	No	No				bek, eit,), 13- 01
Similar to OECD 301 B, modified test concentrations, additional test media	ready	CO_2 evolution	Activated sludge	30 mg dry weight/l and 100 mg dry weight/l	No	No, but in the second study part nitrogen free test medium was used, thus, Cyanamide served as the only nitrogen source	2 and 30 mg a.s./L, test concentrations related to DOC/l out of acceptable range	56 d, also measured after 28 days	Mean of two replicates19% (28 days)	Matla, YA, Hanstveit, AO (1990), Doc No 713-002; Doc IIIA, 7.1.1.2.1/02

Yes, sodium acetate was added in half of the test samples as an additional source of carbon.

2 and 30 mg/L refers to the 50% aqueous solution of cyanamide, the concentrations of pure cyanamide were therefore 1 and 15 mg/L. Only the 1 mg/L samples were tested with and without the addition of sodium acetate.

19 % degradation reflects only the samples of 1 mg/L pure cyanamide without sodium acetate; the addition of sodium acetate resulted in 100% degradation of pure cyanamide within 13 days.

Table 2 *The results of the chemical analysis of hydrogen cyanamide in the biodegradation test with nitrogen free medium. A and B are parallel bottles.*

nominal conc. hydrogen cyanamide mg.l^{-1}	concentration sodium acetate mg.l^{-1}	% degradation of hydrogen cyanamide days			
		0	13	28	56
1 A		0	6	14	18
1 B		0	10	24	25
1 A	100	0	100	–	–
1 B	100	0	100		

This study could clearly show that under certain circumstances (cyanamide as the only nitrogen source in the presence of sodium acetate as an additional carbon source for the activated sludge), cyanamide is readily biodegradable. However these test conditions do not reflect the ones required according to the OECD Guideline 301B. Therefore the results of this test are not sufficient to proof the ready biodegradability of cyanamide.

5.1.2.1.3 Route and Rate of Degradation in Aerobic Aquatic Systems

Subject: To investigate the degradability of cyanamide under environmentally realistic conditions another study was performed which is not taken into consideration in the CLH report: "*^{14}C-Cyanamide Route and Rate of Degradation in Aerobic Aquatic Systems*" (Völkl, S. 2000).
Explanation: According to the CLP Regulation (EC) No 1272/2008 Annex I §4.1.2.9.2
"[…] a fail in the screening test does not necessarily mean that the substance will not degrade rapidly in the environment. Other evidence of rapid degradation in the environment may therefore also be considered […]. Thus, a further classification criterion is included which allows the use of data to show that the substance did actually degrade biotically or abiotically in the aquatic environment by > 70 % in 28 days. Thus, if degradation is demonstrated under environmentally realistic conditions, then the criterion of 'rapid degradability' is met."

The route and rate of degradation of ^{14}C-Cyanamide was investigated in two equilibrated water/sediment systems according to the European Community Commission Directive 95/36/EC of July 14, 1995 amending Council Directive 91/414/EEC Annex I, 7.2.1, Route and Rate of Degradation in Aquatic Systems as well as OECD Draft Guideline "Aerobic-Anaerobic Transformation in Water Sediment Systems", October 1999 and the EPA Pesticide Assessment Guidelines, Subdivision N, Section 162-4, October 1982.

Short summary of results:
Mineralization played the major role in the disappearance of cyanamide from both aquatic systems (river and pond) whilst its degradation to other metabolites and their incorporation to the organic matter of the sediment were of minor importance.

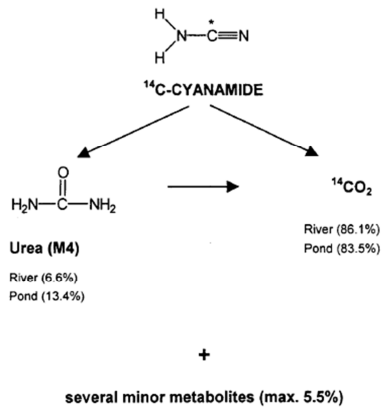
The results show that the degradation proceeded via the formation of urea which was then further degraded to carbon dioxide. The metabolic pathway of cyanamide in water/sediment systems is presented in Figure 22 of the study report, as shown below.

Results summarized in tabular form in more detail:

Type of water/ sediment system	Initial test substance concentra-tion	Total recovery of test substance [% of applied radioactivity]	Degradation rate; represented as % of applied radioactivity detected on certain days [% of applied radioactivity]	Degradation products in the total system (water + sediment) [% of applied radioactivity]	Half-life (DT_{50}) and time to 90% Degradation (DT_{90}) [days]
River	3.3 mg a.i./L	95.4 ± 4.1%	Water phase: Day 6: 47.9% Day 28: 5.9% Extractable from sediments: Day 2 (max): 4.9% Day 28: 0.7% Non-extractable from sediment: Day 28 (max): 11.0% Mineralization (formation of $^{14}CO_2$): Day 28: **86.1%**	Cyanamide: Day 0: 101.2% **Day 12: 0.1%** *Radioactive fractions:* M1 (unknown): Day 2 (max): 5.5% **Day 28: 0.2%** M4 (urea): Day 2 (max): 6.6% **Day 28: n.d.**** M 5***, M6, M7 and M8: Day 6 (max): 0.3% **Day 28: n.d.**	Cyanamide: DT_{50}(w*): 2.3 DT_{50}(t*): 2.5 DT_{90}(w): 7.7 DT_{90}(t): 8.2 M4 (urea): DT_{50}(w): 2.7 DT_{50}(t): 2.9 DT_{90}(w): 9.1 DT_{90}(t): 9.6
Pond	3.3 mg a.i./L	95.7 ± 4.0%	Water phase: Day 6: 69.3% Day 28: 13.2% Extractable from sediments: Day 6 (max): 8.2% Day 28: 1.2% Non-extractable from sediment: Day 21 (max): 7.8% Mineralization (formation of $^{14}CO_2$): Day 28: **83.5%**	Cyanamide: Day 0: 96.7% **Day 28: 0.1%** *Radioactive fractions:* M4 (urea): Day 1(max): 13.4% Day 21: 1.2% **Day 28: n.d.** M1, M2 and M3: Day 6 (max): 5.4% of M1, 1.5% of M2 **Day 28:** **0.6% of M1,** **0.1% of M2**	Cyanamide: DT_{50}(w): 4.3 DT_{50}(t): 4.8 DT_{90}(w): 14.4 DT_{90}(t): 15.8 M4 (urea): DT_{50}(w): 7.5 DT_{50}(t): 8.0 DT_{90}(w): 25.0 DT_{90}(t): 26.7

a.i. = pure cyanamide
*w: Water Phase, t: Total System
**n.d.: not detected or below limit of quantification
***M5: Dicyandiamide

Figure 22: Proposed metabolic pathway of ^{14}C-CYANAMIDE in both aquatic systems (river and pond).



Our investigations on route and rate of degradation of ^{14}C-Cyanamide demonstrate that Cyanamide is rapidly degraded in two different water/sediment aquatic model systems: river and pond. Additionally the major pathway of degradation of ^{14}C-Cyanamide was evaluated by determining the identities and quantitative contributions of significant degradation products. The most prominent degradation product was found to be urea, which itself has been shown before to be readily biodegradable (http://apps.echa.europa.eu/registered/data/dossiers/DISS-9d86bbd9-11ab-41e7-e044-00144f67d249/AGGR-284b33f1-bd47-47a9-86ef-2d17c6f5febd_DISS-9d86bbd9-11ab-41e7-e044-00144f67d249.html#AGGR-284b33f1-bd47-47a9-86ef-2d17c6f5febd).

On day 28 the maximum amount of a degradation product detected - besides CO_2 - was 0.6 % of applied radioactivity in the unknown metabolite M1 in the pond system, whereas full mineralization to $^{14}CO_2$ was found to be 86.1% and 83.5% of applied radioactivity in the river and pond systems, respectively. Incorporation of cyanamide or its metabolites into the organic matter of the sediment played a minor role. The non-extractable radioactivity increased with time up to a maximum of 11.0% and 7.8% of applied radioactivity after 28 and 21 days of incubation for the river and pond systems, respectively.

5.1.3 Summary and discussion of degradation

Rapid degradation of cyanamide on soil surface could be demonstrated with a photolytic half-life of 1.45 days. Cyanamide was also subject to phototransformation in water but not in air.

Regarding biodegradability, cyanamide was shown not be readily biodegradable according to the definition of OECD guideline 301 E. A first hint on its biodegradability could however be shown in a modified version of the OECD Test 301 B. In this test 100 % of applied cyanamide was degraded within 13 days when cyanamide served as the only nitrogen source in the presence of an additional carbon source for the activated sludge.

Additionally, rapid degradation of cyanamide could clearly be demonstrated under environmentally realistic conditions in two aerobic water/sediment model systems. In both systems within 28 days cyanamide was degraded by 99.9 %. Of the applied radioactively marked cyanamide > 83 % could be shown to be fully mineralized to CO_2 within 28 days either directly or via the intermediate metabolite urea. On day 28 all of the identified known or unknown metabolites were either incorporated into the organic matter of the sediment or present in the water phase by less than 0.6%.
Cyanamide is therefore considered to be rapidly degradable according to the CLP Regulation (EC) No 1272/2008, Annex I sections 4.1.2.9.2 and 4.1.2.9.3.

5.2 Environmental distribution
5.2.1 Adsorption/Desorption

Subject: There is a typing error in Table 158 of the CLH report that should be corrected. This typing error doesn't affect the overall results but should be mentioned to avoid confusion.
Explanation: Cyanamide was hardly adsorbed to soil in this test. The result is presented in the study report of the reference which is mentioned in the CLH Report and it is also described in the text of the CLH Report following Table 158. Nevertheless, in table 158 adsorption of cyanamide to soil is stated to be > 2%. The symbol ">" must be replaced by "<" because adsorption was less than 2% in every tested soil.

For more detailed explanation please compare the following copies of the CLH report and the original study report:

Table 158: Results of Adsorption/desorption screening test

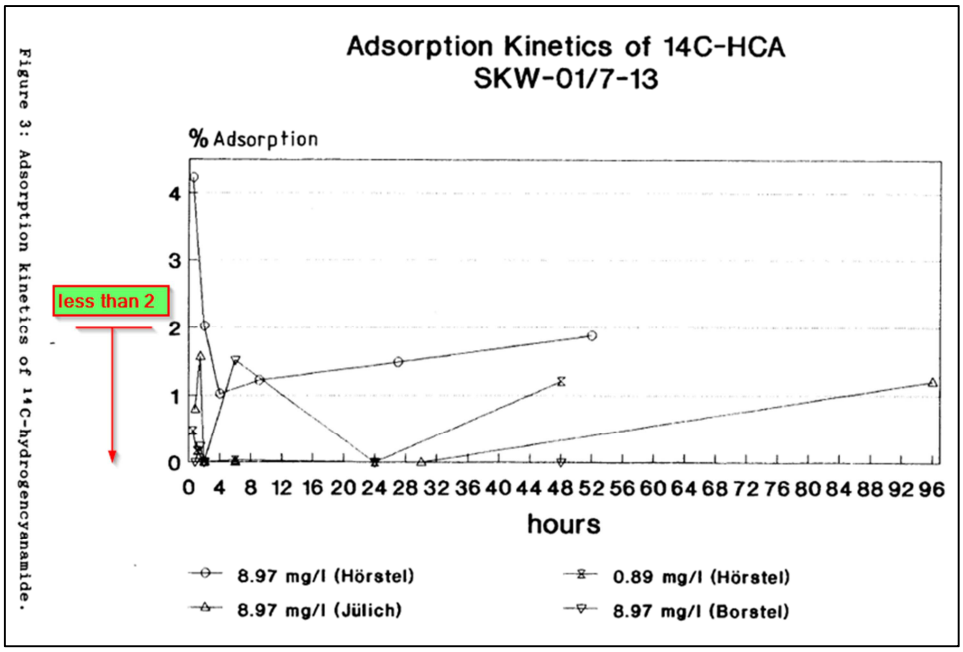
Method/ Guideline/ Tested soils	Classification	Adsorbed a.s. [%]	K_d[1] (ml/g)	K_{OC} (ml/g)	Reference
OECD 106					Rüdel, H. 1990
Hörstel (8.97 mg/L)	Acidic sand	> 2	0.092	6.81	
Hörstel (0.89 mg/L)	Acidic sand	> 2	0.059	4.35	
Jülich	Loamy silt	> 2	0.060	6.34	
Borstel Ap	Slightly acidic silty sand	> 2	0	0	
Arithmetic mean				4.38	

[1] K_d = Distribution coefficient

The adsorption/desorption characteristics of Cyanamide on three soils were investigated. In all samples the adsorption of Cyanamide to soil was low. After 48 hour, the adsorption was less than 2 % for all soils and both concentrations. Linear distribution coefficients (K_d) were calculated from the mean ratios of adsorbed test substance to test substance in the aqueous phase. For the soil Hörstel adsorption coefficients, normalised to organic carbon K_{oc}, of approximately 6.81 mL/g and 4.35 mL/g were calculated for the high (8.97 mg/L) and low (0.89 mg/L) test concentrations, respectively. For the soil Jülich the K_{oc} value was calculated to be 6.34 mL/g, whereas with the soil Borstel Ap no adsorption was detectable. In compliance with the guideline, the desorption part of the study was not performed, because significant adsorption (approximately 25 % or less) had not occurred. The derived K_{oc} –values indicate, that Cyanamide will not be adsorbed in soils and point towards a high mobility potential of Cyanamide in soil.

a.s. = pure cyanamide; all test substance values refer to pure cyanamide

In all cases the adsorption was low. After 48 h, a maximum of adsorption was observed with 1.88 % for the soil Hörstel (acidic sand; 1.35 % organic carbon). With the other soils the adsorption was nearly zero. The relatively high adsorption at study start which was observed especially with the soil Hörstel was probably due to a not homogenous distribution of the test substance in the solution at study start.



5.3 Aquatic Bioaccumulation

No comment on this section.

5.4 Aquatic toxicity

Subject: The chronic (21d) NOEC value of 0.05 mg pure cyanamide/L for *Daphnia magna* needs to be corrected to 0.1044 mg pure cyanamide/L.
Explanation: For detailed explanation please see section 5.4.2.2 of this comment.

5.4.1 Fish

5.4.1.1 Short term toxicity to fish

No comment on this section

5.4.1.2 Long term toxicity to fish

No comment on this section

5.4.2 Aquatic invertebrates

5.4.2.1 Short term toxicity to aquatic invertebrates

No comment on this section

5.4.2.2 Long term toxicity to aquatic invertebrates

Two major problems exist, regarding the study "*Chronic Toxicity of Hydrogen Cyanamide to Daphnia magna Under Flow-Through Test Conditions*" (Murrell, H.R.; Leak, T. 1995). These two major problems are discussed within subjects 1 and 2.
Subject 1: Due to a slight typing error when transferring the raw data to the statistical calculation sheets some study results need to be corrected.
Subject 2: In contrast to what is mentioned in the CLH Report, the study results of this study refer to pure cyanamide, not to the 50 % aqueous solution of cyanamide.
Subject 3: In contrast to what is mentioned in the CLH Report, the parameter length was not significantly affected in all concentrations compared to control. The NOEC length should therefore be removed from table 165 of the CLH Report and also from the text following this table.
Subject 4: As the determined NOEC for cyanamide appears to exactly reflect the classification limit between two categories, the mean measured concentrations should be calculated in greater detail.
Subject 5: Even though the CLH Report refers to this study (Murrell, H.R.; Leak, T. 1995) it is not mentioned in the reference list of the CLH Report.

Explanation to subject 1: The endpoint "number of offspring" (YAD=young per adult per reproduction day) showed significant changes with increasing concentrations of cyanamide. Of all tested endpoints YAD was the only endpoint that showed significant dose dependent effects of exposure to cyanamide (for more details see also explanation to subject 3).
Due to the above mentioned typing error, the mean value of YAD of the untreated control has to be corrected from 10.83 to 11.33 YAD. As a consequence, the first significantly changed YAD value is no longer 8.54 but 9.64. This means that 0.1 mg/L is now the highest concentration tested with no significant effect on *Daphnia magna*, meaning NOEC = 0.1 mg/L. Whereas the former NOEC of 0.21 mg/L now becomes the LOEC, because it was the lowest concentration at which a statistically significant effect to *Daphnia magna* was observed.
This issue is illustrated in the following modified copy of the study report:

TABLE VI

Summary of Data and Statistical Analyses for a 21-Day Chronic Toxicity Study of *Daphnia magna* Exposed to Hydrogen Cyanamide

Mean Measured Concentrations	Mean % Survival	Mean YAD	Mean Length (mm)	Mean Weight (mg)	1st BRD day
Control	100.0	~~10.83~~ 11.33	4.74	0.97	7.00
0.023 mg/L	100.0	10.91	4.53*	0.88	7.00
0.049 mg/L	97.5	11.50	4.63	0.91	7.00
0.10 mg/L =NOEC	95.0	10.49	4.68	0.97	7.00
NOEC 0.21 mg/L =LOEC	100.0	9.64 9.64*	4.61	0.98	7.00
LOEC 0.41 mg/L	97.5	8.54*	4.53*	0.92	7.00

Data subjected to a one-way analysis of variance (ANOVA) and Dunnett's multiple means comparison test. Significant differences to the control are noted with a *.

Key to abbreviations; YAD = Young/adult/reproduction day, and BRD = Days to Brood

Red text is corrected | Black text refers to result before correction

Explanation to subject 2: As already mentioned in section 5.1.2.1.1 of this comment, throughout several tests that have been performed with the test substance cyanamide there appears to be confusion with the actual concentration of active substance used in the test. This confusion results from the fact that some tests have been performed with pure hydrogen cyanamide whereas other tests have been performed with a 50% aqueous solution of hydrogen cyanamide.
Unfortunately, at first glance the study report of the study on chronic toxicity of cyanamide to *Daphnia magna* gives no clear advice as to which substance these values and also the test results refer, to the 50% aqueous solution or to pure cyanamide. The attached position paper (Nickel G., 2014) summarizes the analysis of the study report as well as the raw data package to show that the determined study results refer to pure cyanamide.
Table 165 and the corresponding text in the CLH Report should therefore be corrected as shown below in the "Explanation to subject 3".

Explanation to subject 3: When discussing the parameter length of daphnids it is said in the CLH Report that "*The parameter length was significantly affected in all concentrations compared to control and therefore NOEC length is < 0.023 of a 50 % (w/w) aqueous solution of Cyanamide/L, this corresponds to < 0.0115 mg a.s./L. As the mean weights were not significantly different to the control at any test concentration and as reproduction is regarded as most relevant parameter for the survival*

of a population, the appropriate effect value for assessment is the NOEC for reproduction of 0.05 mg a.s./L."

However it is clearly stated in the study report that the length of daphnids was not significantly affected in a dose dependent manner, as it can be seen in the following copy of the study report:

> The 0.023 and 0.41 mg/L test levels were significantly different when compared to the control for adult length. The length was not significantly different in the other concentrations compared to the control. The mean lengths for the control and levels one through five were 4.74, 4.53, 4.63, 4.68, 4.61, and 4.53 mm, respectively. The data for the 0.023 mg/L test level were considered aberrant because it did not follow a normal dose-response pattern. Also, the 0.023 mg/L test level had the second highest Young/Adult Reproduction Day (YAD) mean of all the test levels and control.

Taking into account subjects 1, 2 and 3, table 165 of the CLH Report needs to be corrected as follows:

Table 165: Long-term toxicity of Cyanamide to invertebrates

Guideline/ Test method	Species	Endpoint / Type of test	Exposure		Results [mg a.s./L]			Remarks	Reference
			design	duration	NOEC	EC50	LC100		
U.S. EPA-FIFRA, 40 CFR, Section 158.490, Guideline 72-4 (b)	*Daphnia magna* (water flea)	survival~~gro wth~~ **reproduction**	flow-through	21 d	- ~~< 0.0115~~ ~~0.05*~~ 0.1 mg/L* (YAD)	~~> 0.2~~ >0,41 mg/L (survival); No daphnids died at the highest concentration tested	-	results based on mean measured conc.	Murrell, H.R. and Leak, T. (1995)

* The evaluation of the applicant resulted in different effect values. However, there was a mistake in the raw data.

Accordingly the text following table 165 in the CLH Report needs to be corrected in that way, that "50% (w/w) aqueous solution of cyanamide" is replaced by "pure cyanamide" and that the section concerning the "NOEC length" should be removed.

Explanation to subject 4: As described above, the determined NOEC for the effects of cyanamide on *Daphnia magna* is 0.1 mg pure cyanamide/L. This value lies exactly on the border line of classification as it can be seen in the following copy of Regulation (EC) 1272/2008:

Category Chronic 2:

Chronic NOEC or EC_x (for fish)	> 0,01 to ≤ 0,1 mg/l and/or
Chronic NOEC or EC_x (for crustacea)	> 0,01 to ≤ 0,1 mg/l and/or
Chronic NOEC or EC_x (for algae or other aquatic plants)	> 0,01 to ≤ 0,1 mg/l.

Category Chronic 3:

Chronic NOEC or EC_x (for fish)	> 0,1 to ≤ 1 mg/l and/or
Chronic NOEC or EC_x (for crustacea)	> 0,1 to ≤ 1 mg/l and/or
Chronic NOEC or EC_x (for algae or other aquatic plants)	> 0,1 to ≤ 1 mg/l.

Among all species tested *Daphnia magna* was the most sensitive species and the only species that would potentially cause classification of cyanamide into one of the aquatic toxicity categories. In turn among all parameters tested for chronic effects of cyanamide on *Daphnia magna* only one parameter showed effects at all. The affected parameter was reproduction. The determined NOEC was 0.1 mg pure cyanamide/L the corresponding LOEC was 0.21 mg pure cyanamide/L, which is more than twice as much as the NOEC.
It appears that the only reason why the NOEC is not > 0.1 mg pure cyanamide /L is that the mean measured concentration of cyanamide in the test was expressed as exactly 0.10 mg pure cyanamide /L. Therefore we calculated the mean measured concentration in greater detail as shown below in the modified copy of the study report:

TABLE II

Measured Concentrations of ^{14}C-Hydrogen Cyanamide Equivalents During the 21-Day Chronic Toxicity Study with *Daphnia magna*

		Measured Concentration (mg/L)					
Sample	Control	Level 1 (0.025)[a]	Level 2 (0.050)[a]	Level 3 (0.10)[a]	Level 4 (0.20)[a]	Level 5 (0.40)[a]	Diluter Stock (400)[a]
Day -5[b]	<MQL[c]	0.0222	0.0476	0.102	0.197	0.400	393
		0.0234	0.0474	0.103	0.208	0.398	
Day 0	<MQL[c]	0.0240	0.0455	0.103	0.202	0.405	402
		0.0240	0.0481	0.108	0.201	0.406	
Day 4	<MQL[c]	0.0243	0.0485	0.101	0.201	0.404	392
		0.0243	0.0507	0.102	0.208	0.407	
Day 7	<MQL[c]	0.0204	0.0468	0.0998	0.195	0.390	403
		0.0204	0.0476	0.0985	0.198	0.402	
Day 11	<MQL[c]	0.0277	0.0569	0.123	0.241	0.473	---
		0.0261	0.0567	0.122	0.249	0.469	
Day 12	<MQL[c]	0.0230	0.0472	0.102	0.201	0.397	---
		0.0228	0.0489	0.101	0.205	0.404	
Day 13	<MQL[c]	0.0238	0.0475	0.102	0.197	0.392	---
		0.0232	0.0467	0.101	0.206	0.394	
Day 14	<MQL[c]	0.0231	0.0465	0.104	0.197	0.403	396
		0.0221	0.0464	0.106	0.204	0.407	
Day 21	<MQL[c]	0.0225	0.0461	0.0988	0.198	0.403	393
		0.0222	0.0488	0.102	0.197	0.403	
Mean[d]	---	0.023	0.049	0.10 =0.1044	0.21	0.41	400
SD	---	0.0018	0.0034	0.007	0.016	0.024	5
CV, %	---	7.8	6.9	7.0	7.6	5.9	1.3
Recovery, %	---	92	98	100	105	103	100

[a] Nominal concentration(mg/L).
[b] Pre-exposure concentrations, with the exception of diluter stocks, are not included in the summary statistics.
[c] MQL = Minimum Quantifiable Limit; mean MQL for the study = 0.0052 mg/L.
[d] Value rounded to 2 significant figures.

The exact NOEC (YAD) therefore is 0.1044 mg pure cyanamide/L.

In summary the outcome of the study "Chronic Toxicity of Hydrogen Cyanamide to *Daphnia magna* Under Flow-Through Test Conditions" was the following:
Of all evaluated parameters (survival, reproduction, length, weight, days to first brood, sublethal effects) the only parameter that showed any dose dependent effect was reproduction. The substance "pure cyanamide" was found to have a LOEC (YAD) of 0.21mg pure cyanamide/l and a NOEC (YAD) of 0.1044 mg pure cyanamide/l.

Tables 161 and 165 of the CLH Report should therefore be corrected as follows:

Table 161: Summary of relevant information on aquatic toxicity

Method	Remarks	Results	Reference
Acute toxicity test to *Daphnia magna*	OECD 202	EC_{50} = 3.2 mg a.s./L	Adema, M.M. (1983)
Long term toxicity test with *Daphnia magna* (reproduction)	U.S. EPA-FIFRA, 40 CFR, Section 158.490, Guideline 72-4 (b).	NOEC = ~~0.05 mg~~ a.s./L 0.1044 mg/L	Murrell, H.R. and Leak, T. (1995)

Table 165: Long-term toxicity of Cyanamide to invertebrates

Guideline/ Test method	Species	Endpoint / Type of test	Exposure		Results [mg a.s./L]			Remarks	Reference
			design	duration	NOEC	EC50	LC100		
U.S. EPA-FIFRA, 40 CFR, Section 158.490, Guideline 72-4 (b)	*Daphnia magna* (water flea)	survival~~gro~~ ~~with~~ **reproduction**	flow-through	21 d	- ~~< 0.0115~~ ~~0.05*~~ ~~0.1 mg/L (YAD)~~ 0.1044 mg/L* (YAD)	~~> 0.2~~ >0,41 mg/L (survival); No daphnids died at the highest concentration tested	-	results based on mean measured conc.	Murrell, H.R. and Leak, T. (1995)

* The evaluation of the applicant resulted in different effect values. However, there was a mistake in the raw data.

5.4.3 Algae and aquatic plants

Subject: There is a typing error in the text following table 166 of the CLH Report. This typing error doesn't affect the overall results but should be mentioned to avoid confusion.

Explanation: It is mentioned in chapter 5.4.3 of the CLH report as well as in the summary of the study report that exponential growth of algae was observed for up to 72h, whereas growth declined between 72 and 96h. The CLH Report concludes that "*therefore effect values related to the 72-h-growth period are regarded relevant.*"

Table 166 displays correct values, nevertheless, to avoid confusion we would like to mention that there is a typing error in the text following table 166:

The typing error is marked with yellow colour in the following copy of the corresponding text in the CLH Report:

nominal concentrations of the test item. Between 72 and 96 h there was no exponential growth of the algae, therefore effect values related to the 72-h-growth period are regarded relevant. The 72 h effect values contained in the table above were calculated by the CA. The relevant endpoint for the assessment is the growth rate with an ErC_{50} of 32.5 mg of a 51.1% (w/w) aqueous solution of Cyanamide/L, this corresponds to 14.7 mg a.s./L. The NOErC amounts 5.0 mg of a 51.1% (w/w) aqueous solution of Cyanamide/L, this corresponds to 2.6 mg a.s./L.

The correct ErC_{50} (72h) would be 28.8 mg/L, as it can be seen in the following copy of the original study report:

Parameter	Growth rate µ (mg/l)			
	24 h	48 h	72 h	96 h
EC50 95%-conf. limits	26.1 17.8-68.5	24.4 16.9-n.d.*	28.8 23.1-41.5	32.5 23.5-84.1
EC10 95%-conf. limits	7.4 3.9-10.2	9.9 3.0-14.0	9.9 8.0-11.5	12.9 8.9-16.2
EC90 95% conf. limits	91.9 43.5-n.d.*	59.9 30.6-n.d.*	84.3 54.4-n.d.*	81.9 44.1-n.d.*
NOEC	5	5	5	5
LOEC	10	10	10	10

Nevertheless, all following values in the text of the CLH Report are correct as well as the values in table 166 of the report.

5.5 Comparison with criteria for environmental hazards (sections 5.1 - 5.4)

Taking into account the information contained in the CLH Report as well as the information contained in this comment, one can summarize the ecotoxicological characteristics of cyanamide as follows:

5.1 → Degradation: not 'readily biodegradable' by definition but 'rapid degradability' could be demonstrated under environmentally realistic conditions; hydrolytically stable; potentially photodegradable in water and soil but presumably not in air.
5.2 → Environmental distribution: high mobility potential in soil
5.3 → Aquatic Bioaccumulation: log K_{ow} = -0.72 (meaning log K_{ow} < 4; low bioaccumulation potential)
5.4 → Aquatic Toxicity: not acutely toxic (EC_{50}/LC_{50} > 1 mg/L), but harmful to aquatic life with long lasting effects (NOEC (21d) = 0.1044 mg/L; LOEC (21d) = 0.21 mg/L; EC_{50} (21d) >0.41 mg/L)

5.6 Conclusions on classification and labelling for environmental hazards (sections 5.1 - 5.4)

For short-term and long-term toxicity of cyanamide to aquatic organisms, *Daphnia magna* was the most sensitive species. The values used for classification therefore refer to *Daphnia magna*.

Acute aquatic toxicity:
The effect level for acute aquatic toxicity category 1 with EC_{50} ≤ 1 mg /L was not reached for cyanamide. The lowest acute value found was an EC_{50} of 3.2 mg pure cyanamide/L for *Daphnia magna*. Therefore no classification as "Aquatic Acute 1; H400" is required.

Chronic aquatic toxicity:
Cyanamide was found to be rapidly degradable according to Regulation (EC) 1272/2008, Annex I, Section 4.1.2.9.
The chronic NOEC (YAD) for *Daphnia magna* was 0.1044 mg pure cyanamide/L which is slightly higher than the limit value of 0.1mg/L for the classification of rapidly degradable substances as "Aquatic Chronic 2".
Accordingly cyanamide is to be classified as "Aquatic chronic 3" (Category Chronic 3: Chronic NOEC or ECx (for crustacean) >0.1 to ≤ 1 mg/L).

Summary:
Due to the above mentioned reasons cyanamide is to be classified as **Aquatic Chronic 3; H412** according to CLP-Regulation (EC) 1272/2008. "Harmful to aquatic life with long lasting effects."

References not cited in the CLH report (unpublished studies assessed by CA Germany under BPD):

Burri, R. (2001): Photolysis of 14C-Cyanamide on Soil Surface Under Laboratory Conditions. Research and Consulting Company, Itingen, Switzerland, Report No.: 744930, GLP, unpublished, Doc. No.: 724-002

Nickel, G. (2014): Clarification on the identity of the test substance and on resulting NOEC.SCC, Bad Kreuznach, Germany. Will be uploaded as non-confidential attachment

Schmid, J. (1990): Aerobic Soil Metabolism of 14C-Cyanamide.ABC Laboratories, Columbia, USAReport No.: 38438GLP, unpublished, Doc. No.: 722-001

Völkl, S. (2000): 14C-Cyanamide Route and Rate of Degradation in Aerobic Aquatic Systems. Research and Consulting Company, Itingen, Switzerland, Report No.: 744952, GLP, unpublished,Doc. No.: 714-002