Committee for Risk Assessment

RAC

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

Annankatu 18, P.O. Box 400, FI-00121 Helsinki, Finland | Tel. +358 9 686180 | Fax +358 9 68618210 | echa.europa.eu

05 June 2015

CLH-O-0000001412-86-67/F

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

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

Chemicals name: **Cyanamide; Carbamonitril**

EC Number: **206-992-3**

CAS Number: **420-04-2**

The proposal was submitted by **Germany** and received by RAC on **8 August 2014.**

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

PROCESS FOR ADOPTION OF THE OPINION

Germany has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **26 September 2014**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **10 November 2014**.

ADOPTION OF THE OPINION OF THE RAC

Rapporteurs, appointed by RAC: **Thomasina Barron and Brendan Murray**

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

The RAC opinion on the proposed harmonized classification and labelling was reached on **5 June 2015** by **consensus.**

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

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard state-ment Code(s)	Suppl. Hazard statement Code(s)	
Current Entry	615-013-00-2	cyanamide; carbanonitril	206-992-3	420-04-2	Acute Tox. 3 * Acute Tox. 4 * Eye Irrit. 2 Skin Irrit. 2 Skin Sens. 1	H301 H312 H319 H315 H317	GHS06 Dgr	H301 H312 H319 H315 H317		
Proposal for RAC	615-013-00-2	cyanamide; carbamonitril	206-992-3	420-04-2	**Add:** Repr. 2 STOT RE 1 Aquatic Chronic 1 **Modify:** Acute Tox. 3 Acute Tox. 3 Skin Corr. 1B Skin Sens. 1B **Remove:** Eye Irrit. 2	**Add:** H361fd H372 (thyroid) H410 **Modify:** H301 H311 H314 H317 **Remove:** H319	GHS08 GHS06 GHS05 GHS09 Dgr	**Add:** H361fd H372 (thyroid) H410 **Modify:** H301 H311 H314 H317 **Remove:** H319		M=1
RAC opinion	615-013-00-2	cyanamide; carbamonitril	206-992-3	420-04-2	**Retain:** Skin Sens. 1 **Add:** Carc. 2 Repr. 2 STOT RE 2 Aquatic Chronic 3 **Modify:** Acute Tox. 3 Acute Tox. 3 Skin Corr. 1 **Remove:** Eye Irrit. 2	**Retain:** H317 **Add:** H351 H361fd H373 (thyroid) H412 **Modify:** H311 H301 H314 **Remove:** H319	GHS08 GHS06 GHS05 Dgr	H351 H361fd H301 H311 H373 (thyroid) H314 H317 H412		

Resulting Annex VI entry if agreed by COM	615-013-00-2	cyanamide; carbamonitril	206-992-3	420-04-2	Carc. 2 Repr. 2 Acute Tox. 3 Acute Tox. 3 Skin Corr. 1 Skin Sens. 1 STOT RE 2 Aquatic Chronic 3	H351 H361fd H301 H311 H314 H317 H373 (thyroid) H412	GHS08 GHS06 GHS05 Dgr	H351 H361fd H301 H311 H314 H317 H373 (thyroid) H412		

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

Test substance

Cyanamide was evaluated as a pesticidal active substance under Directive 91/414/EEC. In this process, it was considered that the technical active substance, cyanamide, is an aqueous solution containing at least 488 g of pure cyanamide per kg solution. The technical active substance was tested under the following product/brand names: Cyanamide L 500, aqueous cyanamide solution, SKW Cyanamide L 500, aqueous hydrogen cyanamide and aqueous cyanamide.

The technical active substance was regarded as identical to the plant protection products (PPPs) ALZODEF (formerly registered in Germany as a herbicide and as a plant growth regulator for sucker control in hops) and DORMEX (registered in various EU countries as a plant growth regulator for breaking bud dormancy). It was concluded that for this reason it is not necessary to distinguish between the technical active substance and the PPPs ALZODEF and DORMEX.

In the studies conducted from the 1970s until 2009, the purity of cyanamide used ranged from about 20% to nearly 100%. In most studies an aqueous solution of cyanamide of approximately 50% was used. Doses were calculated and reported as pure cyanamide.

In the REACH registration dossier, it is stated that "Calcium cyanamide is rapidly and quantitatively converted to hydrogen cyanamide upon contact with water (as was already shown in a report; "Conversion rate of calcium cyanamide (technical grade) to hydrogen Cyanamide", Doc. No. 593-001). Consequently, data obtained from toxicological tests performed with calcium cyanamide are also applicable to hydrogen Cyanamide." (cited from Endpoint study record "420-04-2_cyanamide (calcium cyanamide)_Health surveillance data_1976 (Gfaller, 1976).

A similar bridging statement was included in the dossier submitted under Directive 91/414/EEC , which was accepted during the Plant Protection Products (PPP) peer review process. The dossier submitter (DS) agrees with this bridging concept (at least for non-acute endpoints), and hence studies conducted with calcium cyanamide were used to assess the hazards of cyanamide.

HUMAN HEALTH HAZARD ASSESSMENT

RAC evaluation of acute toxicity

Summary of the Dossier submitter's proposal

The current entry in Annex VI to CLP for acute toxicity is a minimum classification, as Acute Tox. 3 (H301) and Acute Tox. 4 (H312). A classification for Acute toxicity category 3 (H301 and H311) is proposed by the DS based on the findings in the acute oral and dermal toxicity studies reviewed under the PPP process.

Two acute oral studies were assessed in the CLH report. The acute oral LD50 in rats was determined to be 142 mg/kg bw cyanamide in the older study (Engels, 1973). Male rats were particularily susceptible with 4/5 animals dying at 125 mg/kg and 5/5 at 150 and 175 mg/kg bw. All animals that died had severe convulsions prior to death. Mortality mainly occurred during the

first day after administration. No necropsy was carried out. According to the DS, Cyanamide is considered to be toxic via the oral route in this study.

A more recent study was carried out under GLP and in accordance with OECD TG 410. An oral LD50 of 223 mg/kg bw cyanamide was obtained. Signs of intoxication included lethargy, hunched posture, uncoordinated movements, tremors, piloerection and breathing difficulties (Daamen, 1994). It is concluded that this study is reliable as it was performed according to GLP and OECD TG 401. The particular sensitivity of males and the convulsions that occurred in the older study were not seen in this study.

Industry did not agree that the Engel (1973) study should be included in the hazard evaluation and instead considered that only the more recent, guideline compliant study should be used. The DS considered the study by Engel (1973) to be of supplementary value for the purpose of classification and labelling, presumably due to its age and a lack of necropsy examination. However, the results from this study were considered as part of the weight of evidence in the derivation of the LD50. Therefore, the oral LD50 (male and female combined) is considered to be in the range 142 - 223 mg/kg bw.

The dermal LD50 of cyanamide was in the range of 2120 - 3180 mg/kg bw in an old study (van Beek, 1973). In a more recent study, the acute dermal toxicity of an aqueous cyanamide solution was re-examined (Osheroff, 1988). Based on the observed mortality pattern in this study, it was concluded by the DS that the LD50 for the pure active substance cyanamide is 848 mg/kg bw combined for the sexes (901 mg/kg bw in males and 742 mg/kg bw in females).

An inhalation study, using a four hour exposure to 1.0 mg of pure active substance cyanamide/L air (the highest attainable concentration) (99% with MMAD < 3μm), was considered to be supplementary (Kruysse, 1973) due to methodological shortcomings. However, it was considered suitable for determining the LC50 in rats. No mortality or severe injury was observed. Therefore, the LC50 of cyanamide was greater than 1.0 mg/L air and the DS did not propose a classification for that route of exposure.

Comments received during public consultation

A single comment from one Member state competent authority (MSCA) supported the classification proposal for acute toxicity.

Assessment and comparison with the classification criteria

In one non-guideline and one guideline compliant study, the oral LD50 values were estimated to be between 142 and 223 mg/kg bw in rats. These values fall within the CLP criteria for Acute Tox. 3; H301 (Toxic if swallowed; > 50 < LD50 ≤ 300 mg/kg).

One guideline study was considered for dermal toxicity and an LD50 of 848 mg/kg bw (combined for the sexes) was derived. According to the criteria in the CLP Regulation, cyanamide should be classified as Acute Tox. 3; H311 (Toxic in contact with skin).

Rats were exposed by the inhalation route to a mist of technical active cyanamide in a concentration corresponding to the highest technically attainable concentration. No mortality or severe injury was observed at this dose. The LC50 was determined to be greater than 1.0 mg/L air, and considering that no higher concentration could be tested, it was concluded that the criteria for classification via inhalation are not met.

In summary, the RAC agrees with the classification proposal of the DS for acute toxicity in category 3 (Acute Tox. 3) for the oral and dermal route with hazard statement codes H301 and H311, respectively, and no classification for acute toxicity via the inhalation route.

RAC evaluation of specific target organ toxicity – single exposure (STOT SE)

Summary of the Dossier submitter's proposal

According to the DS, in the absence of lethality, no toxicity to a specific organ was observed in acute oral, inhalation or dermal toxicity studies in animals.

Cyanamide exposure via ingestion or inhalation, may occur when the technical active substance is handled improperly. Cyanamide induces vasomotor reactions in humans, known as "Cyanamide Flush", which includes several clinical symptoms e.g. facial flushing, tachycardia, dyspnea, hypotensia, headache, nausea, vomiting, tightness in the chest and sensation of coldness in the extremities. The mechanism of the vasomotor reactions, can be defined as an inhibition of acetaldehyde dehydrogenase. In general these symptoms disappear with no residual effects on general health without specific treatment. In the cases of exposure to larger quantities (in the gram range per day) severe irrritating properties of hydrogen cyanamide to the mucous membranes were also observed. Additional effects such as trembling, convulsions, salivation, danger of aspiration, pains behind the sternum and in the epigastrium, unconsciousness and death can occur.

These effects are most pronounced when in combination with alcohol consumption, and the DS concluded that the effect of a combination of cyanamide and alcohol does not justify classification as STOT SE.

Comments received during public consultation

There were no specific comments on this hazard class during the PC.

Assessment and comparison with the classification criteria

No specific target organ was identified in the acute toxicity studies submitted. Significant signs of systemic toxicity were seen following oral and dermal application such as; lethargy, quick breathing, piloerection, hunched posture, uncoordinated movements, chromodachryorrhoea etc. However, there were no abnormal findings in organs at necropsy. According to the Guidance on the Application of the CLP Criteria (CLP guidance), "STOT SE should be considered where there is clear evidence of toxicity to a specific organ especially when it is observed in the absence of lethality" (Section 3.8.1). Effects observed in animals after acute exposure, as occurred in this case, are considered to be covered by the Acute toxicity classifications.

The specific symptoms of the vasomotor reactions in humans when taken in conjunction with alcohol are well documented; facial flushing, tachycardia, dyspnea, hypotension, headache, nausea, vomiting, tightness in the chest and sensation of coldness in the extremities. These are reversible without residual effects and are not associated with a specific target organ.

The RAC agrees with the DS that classification for STOT SE is not justified.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier submitter's proposal

Animal studies:

Three in vivo studies were performed to investigate the skin irritating potential of cyanamide. The results of these studies were somewhat contradictory. The most recent guideline compliant study, Ligget (1989), with an aqueous hydrogen cyanamide solution (49 % w/w) did not show any irritating potential. In the non-guideline study (similar to OECD TG 404) by Van Beek (1982; using SKW-Cyanamide L 500) very slight to moderate erythema, slight ischemia and slight to severe oedema were observed 4 h after dermal application to albino rabbits (New Zealand White (NZW)). After 52 h, well-defined erythema, slight ischemia, distinct incrustation and very slight or slight oedema were noted. The treated skin areas had a slightly purple colour suggesting the presence of haemorrhage. One week after the treatment period most of the skin surface area showed slight to distinct necrosis. Finally, in the (generally compliant/supplementary) study of van Beek (1984), the ALZODEF dilution, which contained 25 % cyanamide, caused erythema, oedema and some slight scaliness after dermal application to NZW rabbits. The erythema was moderate to severe 4 h after treatment, and 24 h after treatment the erythema was slight in two animals and very slight in four animals. Two rabbits showed very slight ischaemia at the end of the exposure period.

Some dermal effects were noted in the 21-day dermal toxicity study in rats (Murugan et al., 1996). This non-GLP study (conducted according to the 'Gaitonde Committee guideline') was considered unacceptable with regard to sub-chronic toxicity but was considered supplementary with regard to dermal effects only. Following exposure of 6 h/day (for 5 days/week over 3 weeks) to the highest concentration (37.5 mg/kg bw hydrogen cyanamide), severe erythema of the skin and reduction in the fur density around the areas of application were observed. In addition, slight erythema was seen in 4/10 animals following the topical application in a Buehler skin sensitisation assay (Mercier, 1988).

In vitro study:

Further relevant information on skin effects was provided by an in vitro dermal corrosion assay using EpiDerm™ reconstructed skin membranes (Reus, 2011). The DS considered that since a corrosive effect was noted after exposure durations of longer than 3 min and shorter than 1 h, classification into category 1B (H314) was justified.

Human evidence:

A number of studies were summarised in the CLH report which indicated that after dermal exposure severe skin irritation can occur in humans. Most of those affected were observed in occupationally exposed personel involved in the treatment of alcoholism, e.g. paramedics, nursing personnel and chemists. The indications were more consistent with allergic contact reactions than irritation per se and were not considered relevant to dermal irritancy.

Based on the available data, the DS proposed classification as Skin Corr. 1B.

Comments received during public consultation

A detailed comment was received from one MSCA outlining their disagreement with the change of classification from Skin Irrit. 2 (H315) to Skin Corr. 1B (H317). The following argumentation was provided:

1. In the Van Beek (1982) study, after one week of treatment on 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 TG 404 in that scoring was performed at 4 and 52 h, instead of the recommended 24, 48 and 72 h.

2. Supporting *in vitro* data, (Reus, 2011), an EpiDerm™ (Epi-200) study, 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-categorisation as 1B/1C can be made when the viability is ≥ 50% after 3 min AND ≤ 15% after 60 min (OECD, 2014).

The point was made that sub-categorisation as Skin Corr. 1B cannot be done on the basis of in vitro results 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. Skin Corr. 1 is considered to be in line with the CLP Guidance section 3.2.2.4 and step 6a in the decisions logic in section 3.2.2.6.

This argument was accepted by the DS, who in their response to comments (RCOM) concluded that the proposal of Category 1 (H314; Causes severe skin burns and eye damage), consistent with the CLP guidance, can be made on the basis of the van Beek (1982) study where necrosis was observed to the end of the study and the conclusion was supported by the *in vitro* EpiDerm™ study.

Assessment and comparison with the classification criteria

The DS considered that the available data weighed together; i.e. the three *in vivo* dermal irritation studies, the 21-day rat dermal study, the Buehler sensitisation study and the reported human dermal effects showed that cyanamide is at least irritating to the skin. In addition, an *in vitro* EpiDerm™ study contributed to the data set. These data are considered for the proposed classification of cyanamide.

1. Van Beek, 1982:

This study was similar to OECD TG 404 (6 NZW rabbits, 4 hour exposure, except that the observation periods were 4 and 52 hours instead of 24, 48 and 72 h). The test substance was Cyanamide L 500 (a 50% aqeuous dilution of cyanamide) and was used undiluted. The study reporting is considered insufficient. The apparently severe dermal effects remained visible at study termination, one week after dermal application. The findings support the occurrence of full thickness destruction and are consistent with classification as Skin Corr. 1.

2. Van Beek, 1984:

The highest cyanamide concentration tested in this study was an aqueous 25% solution, which caused slight to severe erythema 4 hours after treatment (grade 1-4). However, all animals were recovering by 72 hours (0-2) and all reactions had reversed by day 7. Even though the initial reaction was severe, the scores at 72 hours and the total recovery after 7 days do not support classification.

3. Ligget, 1989:

A 49% aqueous solution was tested in 6 NZW rabbits in an acceptable GLP/guideline study (OECD TG 404). This study was negative for dermal irritation.

4. Reus, 2011:

The test substance was a 50.2% aqueous cyanamide solution. Normal human epidermal keratinocytes derived from neonatal-foreskin (NHRK) were used to form EpiDerm reconstructed

skin membranes. In this system, cyanamide (≈50%) resulted in 85% viability after 3 min and 7% after 60 min, meeting the test criteria for a corrosive substance. According to the OECD TG 431 (Adopted 13 April 2004 or 26 September 2014) the positive result from an *in vitro* skin corrosion study such as that of Reus (2011) cannot differentiate between category 1B and 1C, but supports a classification for Skin corrosion in category 1; H314.

5. Murugan *et al.*, 1996:

The extreme exposure regime in this 21-day rat dermal study, while indicating irritancy potential, is not considered relevant for classification for skin irritation/corrosion.

6. Mercier, 1988:

In the Buehler test, slight erythema was seen in a small number of tested animals following topical application (x3) with a 40% aqueous dilution of the test substance (49% cyanamide). These data are not considered relevant for a skin irritation classification.

Human evidence:

A number of reports indicated that after dermal exposure severe irritation can occur in humans. Most of those affected were occupationally exposed in the treatment of alcoholism, e.g., paramedics, nursing personnel and chemists. The indications are more consistent with allergic contact reactions than irritation *per se* and not considered relevant to dermal irritancy.

Three *in vivo* studies and a recently conducted *in vitro* study were considered relevant to the assessment of dermal corrosion/irritancy of cyanamide. The *in vivo* studies varied with respect to reporting quality, test substance and outcome. Two studies fulfil the CLP criteria for skin corrosion, i.e. the van Beek (1982) study and the *in vitro* EpiDerm study; support is provided by the initial response in the Van Beek 1984 study while the remaining study (liggett, 1989) is clearly negative. The weight of evidence supports classification as corrosive.

The RAC concluded that Skin Corr. 1 (H314) is supported and that sub-categorisation is not possible on the basis of the data available.

RAC evaluation of eye corrosion/irritation

Summary of the Dossier submitter's proposal

The DS proposed to remove the existing classification as Eye Irrit. 2 (H319) in view of the proposed classification as Skin Corr. 1 (H314). Two studies for eye irritation are available, Ligget (1991) and van Beek (1974). Both studies were considered acceptable since there were no major deviations from OECD TG 405. Although the study of van Beek was performed prior to implementation of GLP and the eye was not examined after 1 h, the results are considered acceptable. Both studies show eye irritating properties and are considered valid for the purpose of classification and labelling.

The test substance (49% aqueous cyanamide) was administered to one NZW rabbit in the study of Liggett (1991). Significant corneal opacity (24/48/72 hour mean=2), iridial congestion (mean=0.67) and conjunctival erythema (mean=2 with haemorrhage of the nicitating membrane), conjunctival oedema (mean=2) and discharge were seen. The adverse effects were reversed by day 7.

The test substance (50% aqueous cyanamide) was administered to 6 NZW rabbits in the study of van Beek (1974). Moderate to severe eye irritation consisting of slight (grade 1 in 5/5 animals) to moderate (grade 2 in 1/6 animals) opacity, moderate iritis (grade 1 in 6/6 animals), moderate to severe redness (grade >2 and 2 in 6/6 animals) and moderate (grade 2 in 3/6 animals) to severe (grade 3 in 3/6 animals) swelling of the conjunctiva were seen in all test rabbits after 24 h. Moderate erythema and moderate to to severe oedema were present in 5/6 animals at 72 hours. In addition, after 7 days recovery, slight conjunctivitis was still noted in all animals.

Comments received during public consultation

One MSCA agreed with the removal of Eye Irrit. 2 (H319) because cyanamide is classified as skin corrosive and the risk for severe eye damage is implicit.

Assessment and comparison with the classification criteria

Two studies for eye irritation are available, i.e. Ligget (1991) and van Beek (1974). Both studies are considered acceptable since there were no major deviations from OECD TG 405. Although the study of van Beek was performed prior to implementation of GLP and the eye was not examined after 1 h, the result is considered acceptable. However, in this study, even though the conjunctival reaction was not resolved after 7 days, the study was apparently terminated. While the effect on the conjunctiva was clearly resolving, complete reversibility was not demonstrated. In both studies eye irritating properties of cyanamide were seen and were considered sufficient for the purpose of classification and labelling.

It is also noted that the test substance in both cases was a ≈ 50% dilution of cyanamide, the active substance under review, and therefore will significantly underestimate the effect anticipated in the eye following exposure to 100% cyanamide.

However, as the substance is corrosive to the skin (Skin Corr. 1, H314, according to CLP criteria), specific classification as an eye irritant is not appropriate, because eye damage is already included implicitly in the classification for skin corrosion.

Therefore, the RAC agrees with the DS proposal to remove the classification and labelling for eye irritation from the current entry in Annex VI, CLP.

RAC evaluation of skin sensitisation

Summary of the Dossier submitter's proposal

The DS proposed to classify cyanamide as Skin Sens. 1B instead of the existing classification as Skin Sens. 1. Two studies were evaluated to assess the dermal sensitisation potential of cyanamide.

In a GLP and generally guideline-compliant Magnusson and Kligman (M&K) study (Til, 1982), the test substance (100% cyanamide) was clearly positive with a 100% positive response to 1 and 2.5% dilution challenges (intradermal induction at 10% and topical induction at 5%).

The results of the Buehler study (Mercier, 1988) were less clear-cut. This study was not OECD test guideline compliant because three inductions were used instead of 9. The test substance was 49% aqueous cyanamide. Induction was with a 40% dilution of the test material, not found to be irritating. The same dilution was used for the challenge. Two males and two females showed slight

erythema, which did not correlated with histological evidence of an allergic response, after the challenge.

In addition to animal experiments, medical surveillance data from manufacturing plant personnel were reported (CLH report, 4.12.1.6) where potential sensitising properties of hydrogen cyanamide were considered. There were no cases of confirmed or suspected allergic reactions. Secondly, a number of reports of dermal sensitisation in the general public were considered. To date, three cases of allergic contact dermatitis as a harmful side effect of cyanamide use as an alcohol intake inhibitor had been reported. Allergic skin reactions occurred in 9 cases, mostly through accidental contact of the solution with the skin in staff treating patients with Colme® formulation (alcohol-aversion therapy, 6 g active substance/100 mL). Similar skin reactions were observed in staff handling Colme® on the production site and in the manufacturer's control laboratory. Most of those affected were occupationally exposed in the treatment of alcoholism, e.g., paramedics, nursing personnel and chemists. The indications are consistent with allergic contact reactions. The DS regarded the above human data as very limited evidence that cyanamide causes skin sensitisation in humans.

Comments received during public consultation

One MSCA supported the conclusion of the DS that classification as Skin Sens. 1B (H317) was required on the basis of the M&K assay. They also pointed out that a high induction concentration of 5% precludes the identification of a sensitising potential at doses of < 5%. They questioned whether sub-categorisation is therefore appropriate.

Assessment and comparison with the classification criteria

According to the M&K test (Til, 1982) cyanamide had skin sensitising properties: after dermal application of 100% pure active substance, all Albino Guinea pigs showed a positive response in the challenge test.

According to the method of Buehler (Mercier, 1988), an aqueous solution of 53% w/v cyanamide induced some positive skin reactions after the challenge application (4/20 animals). The results of the Buehler test were considered inconclusive. In contrast, the more sensitive M&K test clearly demonstrated a potential for skin sensitisation.

The RAC notes that based on the results in the M&K study, cyanamide fulfills the criteria in the CLP regulation to be classified as Skin Sens. 1B. However, as it is not possible to assess whether a lower induction dilution would also have met the criteria, the data are not considered sufficient to sub-categorise (Annex 1: 3.4.2.2.1.1, CLP), and therefore classification in Category 1 (Skin Sens. 1; H317) was agreed by RAC. The RAC also considers the evidence of human dermal sensitisation as supportive for this classification.

RAC evaluation of specific target organ toxicity– repeated exposure (STOT RE)

Summary of the Dossier submitter's proposal

The short term toxicity of cyanamide was investigated in the rat (a 28-day study in SD rats and a 90-day study in Wistar rats), two 90-day dog studies, and a 52-week oral gavage study in dogs by the oral route. Additionally, a 21-day dermal rabbit study and a 14-day inhalation Wistar rat study were available. According to the DS, the adverse findings in the rat thyroid were considered

relevant to classify cyanamide as STOT RE 1 (H372), while testicular effects in the dog studies were considered under reproductive toxicity (fertility). A brief summary of the studies is presented below.

1. 28-day oral SD rat study (Osheroff, 1988):

In a short-term oral toxicity study of cyanamide in the rat at doses of 0, 5, 10, 20 or 40 mg/kg bw/d over a period of 28 days, toxicity was characterised by a significant depression in body weight, body weight gain and food consumption at 20 and 40 mg/kg bw/d. A decrease in mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) was seen in males and females at the highest dose as well as an incidental increase of total bilirubin. It was assumed that anaemia was caused by haemolysis. Splenic pigmentation was found in females at doses 10 mg/kg bw/d and greater, and this was also seen in the high dose males (40 mg/kg bw/d). Histopathological change in the thyroid was seen at all dose levels. The low dose alterations at 5 mg/kg bw/d were not considered adverse, whereas more severe changes from 10 mg/kg bw/d and above were toxicologically significant. In females, thyroid effects were observed at 20 and 40 mg/kg bw/d. Thyroid function tests demonstrated increased thyroid stimulating hormone (TSH) as well as decreased thyroxine (T4) at 40 mg/kg. Other histopathological findings were seen at 10, 20 and 40 mg/kg bw/d in the liver of males (bile duct hyperplasia). The LOAEL for cyanamide was considered to be 10 mg/kg bw/d, based on the more significant thyroid effects in males (follicular cell hyperplasia, decreased colloid content and small, densly packed follicles).

2. 90-day oral Wistar rat study (Til *et al*., 1975):

A 90-day oral toxicity study was performed in rats with 0.5, 1.5 and 4.5 mg/kg bw/day of cyanamide (corresponding to 10, 30, 90 ppm; feeding study). There were no mortalities and no clinical signs of toxicity. There was a significant increase in liver weights in males and a significant decrease in thymus weights in females at the highest dose. At 4.5 mg/kg bw/d thyroid effects were seen in males as well in females. The changes in the thyroid were comparable to the effects found in the 4-week study, i.e. small follicular lumens without colloid separated by proliferating epithelial cells and interfollicular cells. Histopathological changes in the thyroid at 1.5 mg/kg bw/d in only one male (1/20) were not considered attributable to cyanamide. Additionally, male rats showed – in contrast to the 28-day study – a slight increase in erythrocyte counts. The LOAEL was 4.5 mg/kg bw/d for the pure active substance cyanamide (equivalent to 90 ppm in the diet) in males and females.

3. 90-day oral dog study (Til *et al*., 1982):

In a 90-day oral study, Alzodef was administered *via* gavage at levels of 0.6, 2 and 6 mg/kg bw/d of active substance cyanamide to 4 male and 4 female Beagle dogs/group. The dogs were about 4 months old at the start of the study. Hypothyroidism was apparent from the thyroid function tests; clear decreases in T3 and T4 were observed in the 90-day dog study from 2 mg/kg bw/d and above, but without histopathological correlates. Absolute and relative testis weight was reduced at 6 mg/kg bw/d. At this dose, one dog showed small testes and the other unilateral cryptorchidism. Histopathological findings in testes (signs of atrophy and/or reduced spermatogenesis) and epididymidis (absence of spermatiozoa) accompanied by reduced testes weights were most pronounced at the highest dose tested (6 mg/kg bw/d). Slight changes in the testes and epididymides found in the lower dose groups were regarded by the DS as unrelated to treatment as the effects were considered to be within the background values. In conclusion, the NOAEL in this study is considered by the DS to be 0.6 mg/kg bw/d based on decreased T3 and T4 in the mid and high dose animals.

4. Supplementary 90-day oral dog study (Til & Beems, 1986):

In a supplementary (no clinical chemistry/haematology/urinalysis) 90-day oral toxicity study with dogs, dose levels of 0, 0.6 and 6 mg/kg bw/d of cyanamide were administered to mature male beagle dogs (12 – 15 months at study start). One of four dogs from the high dose group (6 mg/kg bw/d) had tubular degeneration/depletion in the testes, sloughed germ cells/debris in the epididymis and reduced sperm in the epididymis. Although these changes could be incidental, their possible relationship to cyanamide administration cannot be excluded. The findings of this study were confirmed in the pathology review of Weber and Creasy (2009). In this study, the dogs could be considered to be sexually mature unlike the previous 90-day study. The LOAEL was 6 mg/kg bw/d and was based on retarded body weight gain, reduced food consumption as well as evidence of testicular damage.

5. One-year oral dog study (Osheroff, 1989):

In a 1-year study 0, 0.2, 1 and 5 mg/kg bw/d of cyanamide was administered via oral gavage to 4 male and female Beagle dogs/group (6 - 8 months old at study start). It is concluded that the NOAEL of the 1-year study in dog is 1 mg/kg bw/d based on the significantly reduced body weight/gain in both sexes, the occurrence of significant anaemia in female dogs (reduced red blood cell (RBC) parameters also in males) and significantly reduced T4 in dogs at 5 mg/kg bw/d and reduced (not statistically significant) T3. Whether the testicular findings in one dog were treatment-related could not be excluded with certainty.

6. 21-day dermal rabbit study (Murugan *et al*., 1996):

A 21-day dermal study in rabbits revealed local skin effects at doses 25 mg/kg bw/d and greater after an application of Dormex (a 50 % w/w hydrogen cyanamide formulation). Therefore, the NOAEL for dermal effects was 12.5 mg/kg bw/d. A NOAEL for systemic effects was not derived, since the animals were not sacrificed and examined gross pathologically and histopathologically immediately after the end of treatment, but at the end of a 14-day recovery period. The pathology and histopathology data are therefore not acceptable for evaluation of systemic effects.

7. 14-day inhalation Wistar rat study (Kumar *et al*., 1996):

A 14-day inhalation study revealed reduced body weights and body weight gain at 0.15 mg/L air, which was clearly dose-dependent in females at higher doses. Thus, the NOAEL was below 0.15 mg cyanamide/L air in this study in rats.

The adverse findings in the rat thyroid were considered relevant to classification as STOT RE, while testicular effects in the dog studies were considered under reproductive toxicity (fertility).

Comments received during public consultation

One MSCA did not support STOT RE 1 since they considered that clear functional thyroid impairement was not demonstrated and the dose levels with significant and/or severe effects in the 90-day and 1-year dog studies were clearly above the upper limit for STOT RE 1. These data therefore support a STOT RE 2 (thyroid) classfication.

Assessment and comparison with the classification criteria

The relevant studies and the main significant and/or severe effects observed after repeated exposure in rats and dogs are summarised below.

1. The short-term oral 28-day toxicity study of cyanamide in the SD rat (Osheroff, 1988) was characterised by an increased incidence in follicular cell hyperplasia at doses 10 mg/kg bw/d and greater in male rats. Some marginal effects on colloid were seen already at 5 mg/kg bw/d

in the males. The females were less sensitive. Thyroid histopathological effects at 20 and 40 mg/kg bw/d occurred in conjunction with reduced body weights and body weight gain. At 40 mg/kg bw/d, the mean TSH values were increased by 100 %, whereas T4 concentrations were decreased by 28 % in both sexes compared to control animals.

2. In the supplementary 90-day oral toxicity study (Til *et al*., 1975), at 4.5 mg/kg bw/d, predominantly small follicular lumens without colloid, separated by proliferating epithelial cells and interfollicular cells were seen in the thyroid in both sexes, comparable to the effects found in the 4-week study. Hence, significant effects on the thyroid were observed in males and females of two strains of rats. They have toxicological significance and are considered to be of relevance to human health.

3. In addition, the adverse effects on the thyroid in the rat were supported by observations of hypothyroidism in the 90-day and 1-year dogs studies at doses of 2-6 mg/kg bw/d.

According to the CLP Guidance (November, 2013), adverse effects, considered toxicologically significant in the context of STOT RE classification, include '*changes which have affected the function or morphology of a tissue/organ, or have produced serious changes to the biochemistry or haematology of the organism and these changes are relevant for human health*'. All significant health effects that can impair function, both reversible and irreversible, immediate and/or delayed are included.

The effects in the rat studies are seen within the doses indicated in the criteria for STOT RE (oral, rat: 28-day: ≤ 30 mg/kg bw/d; 90-day: ≤ 10 mg/kg bw/d). However, in line with the Specialised Experts recommendation (CP&L Specialised Experts, 1999; ECBI/49/99 – Add. 1 Rev. 2), the particular sensitivity of the rat to antithyroid substances should be taken into account. In addition, the alterations in thyroid hormone homeostasis (seen in the sub-chronic rat study) did not lead to neoplasia in this gland in the long-term studies. The findings in rats were supported by the observation of altered thyroid function in dogs at relatively low dose levels as well. This was not accompanied by organ weight change or histopathology and the effects seem, at these doses at least, indicative of a compensatory reaction rather than of marked organ dysfunction in the dog. Some evidence was presented that the dog (somewhat) similarly to the rat, may also be more sensitive than humans with regard to levels of circulating TBG. The mechanism proposed is relevant to humans but humans are likely to be significantly less sensitive than rodents and probably also less sensitive than dogs.

Overall, the RAC supports classification as STOT RE 2, based on thyroid effects in the rat and supported by some findings in the dog 90-day study. The conclusion takes into account the particular sensitivity of rats as well as evidence of probable higher sensitivity of dogs compared to humans. The apparent lack of adverse effects on the thyroid from the cyanamide treated human population is also considered as supportive information, bearing in mind the possible confounding aspects of the specific population in question.

In conclusion, RAC disagrees with the DS that cyanamide warrants a classification as STOT RE 1 (H372) but concludes that classification as STOT RE 2 (H373) is justified according to the CLP criteria.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier submitter's proposal

A full battery of mutagenicity studies *in vitro* and *in vivo* were submitted and evaluated by the DS, covering all relevant endpoints. The DS concluded that no classification for germ cell mutagenicity is warranted.

All tests were negative with the exception of two *in vitro* clastogenicity assays in Chinese hamster ovary (CHO) cells and in human lymphocytes. In the assay with CHO cells (Ivett, 1987), a significant dose-dependent increase in cells with aberrations was obtained with and without metabolic activation at the 20-hour preparation interval. A statistically significant increase in numerical chromosomal aberrations (exclusive gaps) was obtained at the maximum concentrations (33.3 µg/mL) without S9 mix and at 33.3 and 333.3 µg/mL in the presence of S9 mix in human lymphocytes (Enninga and van de Waart, 1988). Both assays indicate a clastogenic response of cyanamide *in vitro.*

Three micronucleus assays were carried out in order to investigate the potential of cyanamide to cause chromosomal damage *in vivo*. One assay was performed in rats (male and female Wistar rats), and the other two in mice (male and female ICR mice and Swiss mice, respectively). In the micronucleus assay carried out in the rat (Willems, 1979), only one dose level of calcium cyanamide (153 mg/kg bw) administered *via* gavage was investigated. The dose was administered twice with an interval of 24 h. No increase in micronuclei was obtained. In the two assays carried out in mice (Ivett, 1987 and Menargues *et al*., 1984), the test substance (hydrogen cyanamide and cyanamide Colme® at 6 g a.s/100 mL, respectively) was administered via gavage and no induction of micronuclei was obtained up to the highest investigated dose (350 and 247 mg/kg bw, respectively). Therefore the clastogenic effects observed *in vitro* could not be detected *in vivo* and any concerns raised by the *in vitro* data were removed by the three negative *in vivo* tests.

Comments received during public consultation

One MSCA supported the proposal of the DS for no classification for mutagenicity.

Assessment and comparison with the classification criteria

No human data are available for cyanamide. Cyanamide is not positive in an *in vivo* heritable germ cell mutagenicity tests or *in vivo* somatic cell mutagenicity tests in mammals, hence a classification in category 1B is not justified. Despite the positive *in vitro* results (mutagenicity, clastogenicity), the three respective *in vivo* studies showed a clear negative outcome, hence a classification in category 2 is not required

The RAC agrees with the conclusion of the DS on "no classification" for germ cell mutagenicity.

RAC evaluation of carcinogenicity

Summary of the Dossier submitter's proposal

The DS presented three studies on carcinogenicity in the CLH report, two in mice and one with rats, together with one long-term toxicity study with rats. The DS considered that in the long-term toxicity study with SD rats and in the carcinogenicity study with F344 (Fischer) rats, no tumours

observed at any site in the rats could be associated with the administration of the substance. In the carcinogenicity study with B6C3F1 mice, calcium cyanamide was not considered 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/d of hydrogen cyanamide) there was a slight increase in ovarian granulosa-theca cell tumours in female rats. The Maximum Tolerable Dose (MTD) was exceeded at this dose level, since mortality was significantly affected, body weight was adversely affected and kidney histopathology/kidney disease (chronic cystitis) increased at the intermediate and high dose levels. It was concluded that no treatment related changes in the tumour profile were found up to a dose of 200 ppm (12.1 mg/kg/ bw/d of hydrogen cyanamide).

Cyanamide was not proposed to be classified as a carcinogen by the DS on the basis that the possible relevant and treatment-related increase in granulosa-theca cell tumours in female mice occurred at doses exceeding the MTD.

Comments received during public consultation

One MSCA did not agree with the proposal for no classification for carcinogenicity. This was based on the following observations:

- The incidence of benign and malignant phaeochromocytomas in rats was greater than the normal incidence (see Table 106, p. 137, CLH report).
- There was an increase in hemangiosarcomas in male mice and malignant lymphomas in female mice (see Table 109, p. 139, CLH report).
- There was a slight increase in ovarian granulosa-theca cell tumors observed in the high dose group females (200 and 600 mg/kg bw/d of hydrogen cyanamide; Table 116, p. 145, CLH report).

This MSCA also considered that the argument that carcinogenic effects occurred only at doses above the MTD was not acceptable. In addition, they made the point that risk assessment considerations such as comparison with anticipated human exposure were not relevant to hazard assessment and classification.

Assessment and comparison with the classification criteria

Four studies are relevant to the evaluation of carcinogenicity.

1. Chronic toxicity (91-week) in the rat (Osheroff, 1991):

This study was GLP, guideline compliant and considered acceptable by the Rapporteur Member state (RMS). Twenty rats/sex received aqueous hydrogen cyanamide at 2.5, 7.5 and 30 mg/kg bw/d for the first 16 weeks. Due to poor health status during the first weeks of treatment, the dose was reduced to 1, 2.5 and 7.5 mg/kg bw/d from week 17 to 91.

The pattern of mortalities did not appear related to treatment. Clinical signs of toxicity (tremors, rough hair, hunched posture) diminished after the doses were reduced from week 16 onwards. Body weight gain was significantly adversely affected in rats in the mid (7.5 mg/kg) and high dose (30 mg/kg) groups up to week 16, and in the (reduced) high dose group (7.5 mg/kg) only, from week 17 until termination. T3 was significantly reduced in females from 2.5 mg/kg bw/d and above, and in males at 7.5 mg/kg at termination and T4 was reduced in high dose males only at termination. Decreases in mean terminal brain, kidney, liver and testis+epididymides weight were considered related to the considerably reduced mean body weights at terminal sacrifice. The only treatment-related histopathological findings consisted of decreased colloid in the thyroids of

mid and high dose males and high dose females, consistent with the findings in the sub-chronic studies .

2. Carcinogenicity study in F344 rats (Ulland, 1979):

In this non-GLP, non-guideline compliant study, 50 rats/sex were given 100 and 200 ppm (males) and 400 ppm (females) calcium cyanamide (commercial formulation containing 63 % calcium cyanamide) in the feed for 107 weeks. The control group had 20 rats/sex. Food consumption was not measured, and body weights were measured once a month (except weeks 50-80). Actual dietary intake was therefore not calculated.

The sites of neoplasms observed most frequently were the adrenal and pituitary glands, the thyroid and the testes. The pituitary neoplasms as well as adenomas (and hyperplasias) of the thyroid and interstitial-cell tumours of the testes occurred with comparable frequency in control and exposed rats. Therefore, they are not considered test substance-related. The incidences of adrenal neoplasms are summarised in the table below.

Relevant tumour incidences in Ulland (1979) and NTP HC data

	0 ppm	**100 ppm**	**200 ppm**
	Males		
Cortical Adenoma NTP HC data: 0.9%	0/20 (0 %)	3/49 (6 %)	3/50 (6 %)
Phaeochromocytoma, benign	4/20 (20 %)	10/49 (20 %)	15/50 (30 %)
NTP HC: *Benign (male) 243/996 (24.4%) – mean 24.4% (9.7) range 5/50-23/50*			
Phaeochromocytoma, malignant	0/20 (0 %)	0/49 (0 %)	1/50 (2 %)
NTP HC: Malignant (male) 29/996 (2.9%) – mean 2.9%(3.0) range 0/47 – 1/49			
	Females		
Cortical Adenoma NTP HC: 1.3%	3/19 (16 %)	1/50 (2 %)	7/50 (14 %)
Phaeochromocytoma, benign	0/19 (0 %)	4/50 (8 %)	6/50 (12 %)
NTP HC: Benign (female) 27/993 (2.7%) – mean 2.7%(1.9) range 0/50 – 3/50			
Phaeochromocytoma, malignant	0/19 (0 %)	0/50 (0 %)	1/50 (2 %)
NTP HC Malignant (female) 5/993 (0.5%) – mean 0.5%(0.9) range 0/50 – 1/47			

NTP=National Toxicology Program
HC=historical control
*The one-tailed Fisher exact test and Cochran-Armitage test for linear trend in proportions, with continuity correction was used.

There is an apparent increase in benign pheochromocytomas in both male and female rats. No statistical significance was found, however, the lower number of control animals is noted. In such a case, an analysis of the relevant historical control data is important. This has not been done in the CLH report, other than a reference to the work of the PRAPeR 79 working group (CLH report, 4.10.3, p. 149). Adrenal pheochromacytoma is documented as having a high background incidence in male F344 rats (CLP Guidance v. 4, November 2014: Section 3.6.2.3.2).

Some data found in the published literature were included in the table above. These 20 NTP studies were all conducted in the 7 years up to 1995. An even higher incidence of phaeochromacytoma (31.9% of male and in 5.1% of female rats) is recorded when the 1998

updated NTP HC are considered (Haseman *et al.*, 1998). This publication does not differenciate between benign and malignant.

It is well documented that certain strains of rats are susceptible to development of neoplasia of the adrenal medulla with advanced age and also on administration of xenobiotics (Greaves, 1990). The HC data available indicate that the increase in benign pheochromocytoma in the high dose males may be within the background for this tumour which is common in male F344 rats. The incidence in females (8% and 12%) is however considerably outside the HC (2.7% ± 1.9). The incidence of malignant tumours is within the HC of 2.9% for males and outside the HC for 0.5% for females, although the tumour can occur in female control animals.

The occurrence of cortical adenoma (6%, 6% for males and 2%, 12% for females at 100 and 200/400 ppm, respectively) is considerably outside the NTP HC mean of 0.9% for males and 1.3% for females. Assuming that these HC data from the 1990's are relevant for the Ulland study completed in 1979, they may support the conclusion that relationship to treatment is possible which would justify classification as Carc. 2.

3. Carcinogenicity study in B6C3F1 mice (Ulland *et al.*, 1979):

In this non-GLP, non-guideline compliant study, 50 mice/sex were given 500 and 2000 ppm calcium cyanamide in the feed for 100 weeks. The control group had only 20 mice/sex. Food consumption was not measured, body weights were measured once a month (except weeks 50-80). Actual dietary intake was therefore not calculated.

There was a significant positive dose-related trend in mortality for male mice in this study, (76% survival *vs.* 100% in controls) but females were not affected. The only other effect noted was slightly reduced mean body weights at the high dose.

In male mice, the results of the Cochran-Armitage test indicate a dose-related trend in the incidence of hemangiosarcomas of all sites, but the results of the Fisher exact test revealed no significance for the findings. The tumour incidences are compared to the National Cancer Institute (NCI) historical control data for haemangiosarcoma in B6C3F1 mice and published NTP data (as above) for haemangiosarcoma (all sites; see table above). It is presumed that the NCI data were generated in the same facility as the study under review and are therefore relevant for the purpose of this analysis although the dates are not available. Overall, the incidence in the high dose group is considerably outside both sets of historical control data.

A dose-related trend in female mice was obtained in the incidence of lymphoma or leucaemia. The Fisher exact test established a significantly higher incidence in the high-dose group than in the control group. The tumour incidences were compared to the NCI historical control data for malignant lymphoma in B6C3F1 mice quoted in the study and to the published NTP data for malignant lymphoma in female B6C3F1 mice (19 studies; see table below). The increased incidence in female mice in the high dose females of this study is outside both sets of historical control data. Survival in the females of the high dose group was not affected by treatment.

Relevent tumour incidence in Ulland *et al*. (1979) and NTP/NCI HC data

	0 ppm	**500 ppm**	**2000 ppm**
	Males		
Haemangiosarcomas	1/20 (5 %)	2/50 (4 %)	10/50 (20 %)
NCI 13/323 (4%) with a highest observed incidence of 2/19 (10%). *NTP: Male B6C3F1 mice 53/952 (5.6%) mean (SD) 5.6%(3.5) range 1/50 – 7/50.*			

	0 ppm	500 ppm	2000 ppm
Malignant lymphomas	1/20 (5 %)	4/50 (8 %)	3/50 (6 %)
	Females		
Haemangiosarcoma	0/20 (0 %)	0/46 (0 %)	1/50 (2 %)
Malignant lymphomas	1/20 (5 %)	11/46 (24 %)	18/50 (36 %)
NCI HC: 67/324 (21%).			
NTP (19 studies) 167/953 (17.3%) - mean(SD) 17.5% (7.7) and range 3/51-15/50.			

* The one-tailed Fisher exact test and Cochran-Armitage test for linear trend in proportions, with continuity correction was used.

4. Carcinogenicity study (drinking water) in [CRL:CD-1(ICR)BR] mice (Goodyer, 1990):
In a GLP and guideline (OECD TG 451) compliant study, 70, 200 and 600 ppm hydrogen cyanamide was administered to 60 mice/sex in the drinking water for 104 weeks.

Mortality was increased in mid and high dose females at week 104 (23.3% and 23.3% vs 40.0% survival in controls). Body weight gain was impaired in mice for the first 6 weeks. All groups except the high dose males recovered thereafter. Mean body weight gains were significantly reduced at termination in high dose males only. There was a treatment-related statistically significant reduction in water consumption in the medium and high dose groups of males and females during several periods over the duration of treatment. In the medium and high dose groups there was a dose-related chronic cystitis in the urinary bladder, accompanied in the high dose group by a marginal increase in the incidence and severity of atrophic basophilic tubules in the kidney.

There was an increased incidence of proliferative lesions in the stromal/luteal tissues of the ovary in females of the high dose group, including 8 granulosa-theca cell tumours. The hyperplasias were predominantly of the luteal type, as were the granulosa-theca cell tumours. The eight cases of granulosa-theca cell tumours in the high dose and the six cases seen in the mid dose exceed the background range of 0/51 - 3/51 in 10 control groups from previous carcinogenicity studies conducted at the laboratory. Statistically, there was a significant dose response trend in granulosa-theca cell tumours across the four groups ($p < 0.01$) regardless of whether the analysis included or excluded one equivocal necrotic control case. Pairwise statistical comparison between groups 1 and 4 was significant ($p < 0.05$) if the necrotic control case was excluded, but not significant ($p > 0.05$) if this case was included in the statistical analysis.

It was argued by the authors of the study report that the tumours seen in females from the mid dose level are not relevant for classification as the MTD had been exceeded from the mid dose and above. This was demonstrated by significantly increased mortality, an initial adverse effect on body weight, microscopic change in the the urinary bladder and kidney (high dose) and at the high dose, slight to moderate chronic cystitis. It is noted that the reduced water consumption from the mid dose may have influenced/caused the chronic cystitis.

Comparision with the criteria

Category 1B vs. Category 2
In the absence of any human data, classification in category 1B requires sufficient animal evidence to clearly demonstrate carcinogenicity in well conducted studies. There was a possibly treatment-related increase in benign pheochromocytoma in male and female F344 rats and cortical adenoma in females (not statistically significant) (Ulland, 1979); an increase in haemangiosarcoma in male B6C3F1 mice (positive trend) and of malignant lymphoma in females (statistically significant)(Ulland, 1979); and an increase in granulosa-thecal cell tumour in CD-1

mice (significant trend; p<0.01, significant pair-wise; p<0.05)(Goodyer, 1990). Ovarian granulosa-thecal cell tumours were significantly increased (p<0.01) in the CD-1 mice (Goodyer, 1990). Proliferative lesions were observed in the stromal/luteal tissues of the ovary in females of the high dose in this study. All tumours were outside the ranges for historical control data which were considered in this assessment.

No carcinogenic mechanism is apparent for any tumour and the substance is not genotoxic. For these reasons the criteria for category 1B are considered not to be fulfilled, as the evidence is 'limited' rather than 'sufficient' and category 2 is hence considered appropriate based on an increased incidence in ovarian granulosa-thecal cell tumours in the CD-1 mice and supported in addition by increased incidences above historical control values in several tumour types in both rats and mice.

The RAC does not agree with the DS to not classify cyanamide for carcinogenicity. Instead, RAC concluded that cyanamide warrants classification as Carc. 2 on the basis of 'limited evidence' for carcinogenicity.

RAC evaluation of Reproductive toxicity - Adverse effects on sexual function and fertility

Summary of the Dossier submitter's proposal

The DS proposed to classify cyanamide for effects on fertility and sexual function as Repr. 2 (H361f) on the basis of a number of adverse effects seen in three multi-generation studies of varying quality, and on the basis of observed effects on the testis in two 90-day and one 52-week study in dogs.

1. Two-generation study in CD SD rats (Obach and Rives, 1985 (Valles *et al.*, 1987)):
SD rats received 0, 2, 7 and 25 mg/kg bw of cyanamide (as Colme® formulation: 6 g a.s./100ml). This study was non-GLP and non-guideline compliant because the first generation also had a embryotoxicity segment (half of the dams from the F0 generation were sacrificed at day 13 of pregnancy and examined for implantation sites, numbers of embryos and resorptions). The remaining dams were allowed to litter normally. A number of litters were examined for developmental and behavioural parameters, as were a number of F2 litters.

Embryotoxicity segment: There were no clinical signs of toxicity. Body weight gain in high dose females was significantly reduced. At this dose level, corpora lutea, (pre- and post-)implantation sites and live embryos were significantly reduced (p<0.05).

Summary of findings in the embryotoxicity segment

Dose level (mg/kg bw/d)	0	2	7	25
Females on study (litters examined)	20	20	20	20
Maternal bw gain prior to mating (g)	6.58	4.99	4.47	-0.43*
Maternal bw gain GD 0-13(g)	23.0	19.96	19.06	15.34*
Mean daily food intake (g/rat)	17.5	17.2	16.8	14.5
Mean daily water intake (mL/rat)	25.8	26.7	24.5	27.8
Corpora lutea (mean)	12.9	13.2	14.8	10.5*
Implantation sites (mean)	11.2	11.3	13.8	6.0*

Dose level (mg/kg bw/d)	0	2	7	25
Live embryos (mean)	10.2	10.1	12.5	4.5*
Resorptions (mean)	0.85	0.95	1.0	1.3*
Dead embryos (mean)	0.2	0.2	0.35	0.2
Mean embryo weight (mg)	60.8	66.06	63.15	62.67
Mean placental weight (mg)	88.9	91.1	85.2	91.5
Litters with preimplantation loss > 2	6	5	3	15*
Litters with postimplantation loss > 2	4	1	3	5
Litters with litter size < 3	3	3	0	10*

* statistically significant (p< 0.05)

Two-generation segment: Mean body weight gains were significantly reduced in high dose animals in the F0 animals. Less than half of the F0 dams were pregnant. The number of F1 parental animals was reduced due to the low pup numbers from the F0 mating, and many parameters could therefore not be appropriately assessed due to low parental numbers. Female fertility was affected in the F0 generation at 25 mg/kg bw/d as indicated by a reduced number of corpora lutea and the decreased pregnancy rate. In addition, a high pre-implantation loss and embryolethality were noted, leading to a decrease in live litter size.

Summary of reproductive and litter data

Dose level (mg/kg bw/d)	0	2	7	25	0	2	7	25
	F0				F1			
Females mated	20	20	20	20	20	12	17	6
Females pregnant	20	20	20	9	18	18[1]	19[1]	6
Pregnancy body weight gain (g)	106	130	124	63*	143	161	132	117*
Implantation sites (mean)	11.1	12.1	13.4	5.7*	12.5	13.7	13.0	11.7
Postimplantation loss (mean)	2.0	0.8	1.3	1.3	2.3	0.8	0.8	0.67
Stillbirths (mean)	0.3	0.3	1.0	0.1	0.15	0.25	0.06	0
Live pups (mean)	8.8	11.0	11.2	4.2*	10.1	12.6	12.2	11.0
Litter weight (mean, g)	56	73	69	31*	70	83	77	73
Pup birth weight (mean, g)	6.51	6.78	6.23	7.45*	7.03	6.61	6.48	6.77
Pup weight PND 14 (mean, g)	29.8	30.7	27.7	27.5	30.5	31.4	29.9	28.2
Pup weight PND 21 (mean, g)	45.7	46.8	42.7	43.9	48.5	47.4	45.3	44.0

* statistically significant by ANOVA followed by Duncan test/Kruskal-Wallis test followed by Man-Whitney U -test (p< 0.05)

[1] Numbers as given in the CLH report. RAC however noted that the number of pregnant females indicated are higher than the number of mated females.

2. Two-generation study in Wistar rats (Koeter *et al.*, 1986):

An aqueous solution of cyanamide 49% w/w was administered in the diet (at concentrations of 0, 20, 60 and 180 ppm) to achieve average doses of 0, 1.66, 5.14 and 15.41 mg/kg bw/d (equivalent to approximately 0, 0.81, 2.52, and 7.55 mg/kg bw/d cyanamide; see CLH report, p. 171 for details) over two generations (two matings per generation). The study was GLP, compliant with OECD TG 416 with some deviations that downgraded the study as supplemental.

Body weights pre-mating and mean body weight gains were significantly affected during pregnancy and lactation at the high dose level in both generations (see table below). Mating and

fertility were unaffected with the exception of a reduced litter size in the first pregnancies of high dose F0 and F1 dams (180 ppm, equivalent to 7.55 mg/kg bw/d). Pup weights at birth and at weaning were decreased in each of the F1 and F2 litters of the high dose groups. Neonatal viability was slightly reduced at the high dose only (viability indices of 80-93 % compared with 93-100 % in controls) (CLP report p. 167, Table 129).

Other signs of systemic toxicity were confined to the rats of the high dose groups in both generations (F0 and F1) and consisted of reduced food consumption, lower body weights, and retarded body weight gains.

Food intake and body weight development (2 generations/2 matings).

Dose level (mg/kg bw/d)	**0**	**0.81**	**2.52**	**7.55**
Food consumption (% of control)				
F0 males – pre-mating	100	102	98	91*
F0 females				
– pre-mating	100	94	96	89*
– 1st pregnancy	100	98	97	92*
– 2nd pregnancy	100	96	96	88*
F1 males – pre-mating	100	100	97	86*
F1 females				
– pre-mating	100	99	101	91*
– 1st pregnancy	100	98	97	87*
– 2nd pregnancy	100	101	99	89*
Body weight gain (g)				
F0 males – pre-mating	296.5	289.5	273.4	235.7*
F0 females				
– pre-mating	127.6	119.8	119.5	102.7*
– 1st pregnancy	119.5	120.5	116.2	110.6
– 2nd pregnancy	121.2	118.9	116.0	110.5
– 1st lactation	30.6	27.6	30.3	36.3
– 2nd lactation	13.7	17.1	25.5	28.3
F1 males – pre-mating	284.2	289.6	274.2	241.6*
F1 females				
– pre-mating	132.4	133.0	136.8	122.7*
– 1st pregnancy	111.1	114.2	107.9	91.7
– 2nd pregnancy	110.0	117.5	106.3	98.5
– 1st lactation	20.2	26.1	21.9	13.4
– 2nd lactation	17.2	11.3	15.7	26.7

* $p < 0.05$; ** $p < 0.01$

No testicular findings were recorded in the F0 males at any dose or in F1 males of the control group. There was an increased incidence of F1 males with interstitial cell proliferation at the high dose level and tubular atrophy was described in all treated groups. However, only a subset (25 %) of the animals at risk for testicular lesions has been examined in this study, which may have been a reason for the lack of a dose-response. Considering the testicular effects observed with the substance in repeated dose toxicity studies, the testicular findings are likely to be related to treatment, although a definitive conclusion cannot be drawn.
In addition, the thyroid gland was identified as a target organ based on increased weights and histopathological findings at this dose level (see table below).

Relevent histopathology finding

Dose level (mg/kg bw/day)	0 (control)		0.81		2.52		7.55	
F0	**Males**	**Females**	**Males**	**Females**	**Males**	**Females**	**Males**	**Females**
Liver (g)	15.56	9.22	17.34*	8.92	16.67	9.32	15.26	8.44**
Thyroid (g)	0.026	0.026	0.028	0.026	0.029	0.026	0.031*	0.028
Testes (g)	3.62	-	3.61	-	3.56	-	3.65	-
Liver (g/kg bw)	33.3	32.9	34.7	32.4	33.9	33.8	34.3	32.9
Thyroid (g/kg bw)	0.057	0.093	0.057	0.096	0.060	0.093	0.071**	0.110**
Testes (g/kg bw)	7.84	-	7.31	-	7.31	-	8.24	-
F1	**Males**	**Females**	**Males**	**Females**	**Males**	**Females**	**Males**	**Females**
Liver (g)	16.25	9.34	17.04	9.35	16.52	9.73	15.87	8.59
Thyroid (g)	0.024	0.025	0.025	0.025	0.026	0.025	0.028	0.027
Testes (g)	3.72	-	3.73	-	3.66	-	3.65	-
Liver (g/kg bw)	32.1	33.2	32.5	33.0	33.2	34.2	35.6**	33.1
Thyroid (g/kg bw)	0.048	0.089	0.048	0.089	0.053	0.089	0.063**	0.106*
Testes (g/kg bw)	7.53	-	7.22	-	7.33	-	8.16	-

* statistically significant by Dunett test ($p < 0.05$)
** statistically significant by Dunett test ($p < 0.01$)

The histopathology findings have been confirmed by a recently performed peer review analysis (Weber & Creasy, 2009), who otherwise found the study unreliable,lacking in data and poorly reported in a number of aspects. This criticism of the study was examined thoroughly by the DS who concluded that it was unfounded [and that the study was suitable for the purposes of CLP?]. Overall, there were some indications of embryofoetal toxicity at the highest dose of 7.55 mg/kg bw/d, in the pr esence of some reductions in parental body weight gain and clear effects on thyroid weights. Fertility was not considered clearly affected at this relatively low dose feeding study.

3. Two-generation study in Crl:CD SD rats (Morseth, 1990):

This GLP, guideline study was considered acceptable by the DS. Aqueous hydrogen cyanamide was administered by gavage at doses of 0, 2.5, 7.5 and 30.0 mg/kg bw/d for 12 weeks and the doses were then reduced due to toxicity to 1.25, 3.75 and 15.0 mg/kg bw/d for the remainder of the study.

Cyanamide was systemically toxic when administered by gavage to rats at doses of 15/30 mg/kg bw/d. The slightly lower weight gain (6 %) of the F1 parental males at the mid dose level of 3.75 mg/kg bw/d is not considered adverse. Reproductive performance and fertility was affected in F0 and F1 parental animals at the top dose group, resulting in a low fertility index in both generations and in an increase of total litter resorptions in the F0 generation. Gross pathology and histopathology revealed no adverse treatment-related effects on the testis and this was confirmed by the recently performed peer review (Weber & Creasy, 2009). See table below for details.

Summary of Fertility and litter data of F0 and F1 generations.

F0				
Dose level (mg/kg bw/d)	**0**	**2.5 / 1.25**	**7.5 / 3.75**	**30 /15**
Cohabitated pairs	26	26	26	26
Bw gain (g) F0 males – pre-mating	287	273	252	130*
Bw gain (g) F0 females – pre-mating – pregnancy – lactation	 135 130 -1.4	 133 143 0.6	 119 139 3.0	 70* 111 25
Mated females	26	22	24	23
Not pregnant	6	6	6	11*
Pregnant females	20	20	20	15*
Precoital interval > 4 days (%)	70	86	73	75
Mean duration of pregnancy (days)	22.1	22.1	21.9	22.1
Total prenatal litter loss	0	0	0	3
Live litters	20	20	20	12↓
Live litter size at birth	12.85	14.75	14.40	10.67↓
Live litter size on PND 4	13.00	13.26	12.90	8.33*
Pup viability index PND 4	92	83**	88**	84**
Litters with pup mortality > 2 (day 1 - 4)	0	6 (30 %)	6 (30 %)	3 (25 %)
Weaning index (of pups alive on day 4)	92	85	87	88
Male pup weight at birth (g)	6.75	6.12	5.95	6.08
Female pup weight at birth (g)	6.40	6.2	5.7	5.7*
Male pup weight at weaning (g)	51.1	47.6	43.3*	41.5*
Female pup weight at weaning (g)	49.4	47.7	40.2**	41.5*

F1				
Dose level (mg/kg bw/d)	**0**	**1.25**	**3.75**	**15**
Cohabitated pairs	26	26	26	26
Mated females	25	24	26	23
Bw gain (g) F1 males – pre-mating	486	465	459	397*
Bw gain (g) F1 females – pre-mating – pregnancy – lactation	 231 119 1.4	 243 129 -7.5	 236 126 6.6	 208* 94 27
Mated females	26	22	24	23
Not pregnant	2	5	3	7
Pregnant females	24	21	23	19
Precoital interval > 4 days (%)	92	88	85	72
Mean duration of pregnancy (days)	21.8	22.0	21.9	22.0
Total prenatal litter loss	0	0	2	2
Live litters	23	20	21	17
Live litter size at birth	12.65	13.15	14.71*	11.53
Live litter size on PND 4	11.65	11.20	12.75	9.24
Pup viability index PND 4	93	87*	82**	81**
Litters with pup mortality > 2 (day 1 - 4)	2 (9 %)	3 (15 %)	7 (33 %)	4 (24 %)
Weaning index (of pups alive on day 4)	87	81	87	97
Male pup weight at birth (g)	6.47	6.58	6.48	6.25
Female pup weight at birth (g)	6.06	6.14	6.11	5.91

F1				
Dose level (mg/kg bw/d)	**0**	**1.25**	**3.75**	**15**
Male pup weight at weaning (g)	44.8	43.1	43.8	42.6
Female pup weight at weaning (g)	43.3	42.7	41.2	39.9

*p<0.05; **p<0.01
PND=postnatal day

Substance-induced developmental toxicity was observed for high dose F1 and F2 pups in the form of lower litter size, birth weight and body weight gain. Based on the percentage of total pups affected, a reduced survival during early postnatal life was seen for F1 and F2 neonates in all dose groups. A litter-based assessment showed for both generations that the number and percentage of litters affected by mortality of two or more neonates increased in all cyanamide dose groups as compared to the respective control groups (F1: 2/20, 7/20, 7/20, 5/12; F2: 2/23, 5/20, 7/21, 6/16). Substance-induced morphological alterations were not seen on external and visceral inspection in any of the F1 or F2 pups.

The highest dose tested caused significant maternal toxicity. At this dose level, fertility was adversely affected and there was clear evidence of embryotoxicity. Postnatal development appeared to be adversely affected at all dose levels.

In summary, reduced fertility (in the presence of parental toxicity) was observed in two two-generation reproduction studies in SD rats (Vallés *et al*., 1987) and in Crl:CD BR rats (Morseth, 1990). In these studies cyanamide was administered via gavage. It is difficult to compare results from gavage and dietary studies due to the very different exposure situation. However, in a third two-generation study in Wistar rats with dietary administration of cyanamide, a slightly reduced litter size in the first pregnancies of high dose F0 and F1 dams was observed (Koeter *et al*., 1986) which may have been related to treatment. The dose levels in this study were significantly lower compared to the other studies. Significant embryotoxicity was also demonstrated in all three studies (less in the dietary study at lower dose levels).

Adverse effects on the testis
The proposal for fertility classification is also based on the evidence of testicular toxicity in three dog studies, see below, further detailed under the STOT RE section of this opinion.

4. <u>90-day dog study (Til *et al*., 1982):</u>
See STOT RE for further details.
When evaluating the toxicological significance of the findings in the testis, the DS took into consideration additional control data and aspects presented by the study authors Til *et al*. (1982), the registrant (Woutersen & Bruijntjes, 2005) and the pathology peer review of Weber and Creasy (2009). Weber and Creasy (2009) pointed out that in young 4-5 month old male beagles spermatogenesis has not yet begun and the animals would be completely immature at study start. The authors reported that the characteristics of immature and peripubertal testes are indistinguishable from the degenerative changes that occur with testicular toxicants and considered that it is usually not possible to evaluate testicular toxicity reliably in animals of this age. They did, however, agree that there appeared to be a dose-related increased severity in the effects with respect to the testis weight, the reduction in spermatogenesis and the presence of spermatozoa in the epididymis. The authors concluded that there might be a delay in sexual maturation in the cyanamide treated animals.

The DS in evaluating the toxicological significance of these findings, took into account that dogs less than eight months of age have high incidences of hypospermatogenesis, lower testicular

weights, and incomplete filling of epididymal tails with sperm, all compatible with immaturity. In this study, the more severe changes in testes and epididymidis in the high-dose group were considered related to the administration of cyanamide, whereas the slight changes found in the lower dose groups are regarded as within the background.

5. Supplementary 90-day dog (Til & Beems, 1986):
See STOT RE for details.

6. One-year dog study (Osheroff, 1989):
See STOT RE for further details.
Two high dose dogs had moderate diffuse acute (neutrophilic) inflammatory infiltrate of the epididymis, atrophy of the prostatic acini, reduced numbers of spermatids in the testes and reduced sperm in the epididymis. This inflammatory lesion was considered by the reviewers to be caused by canine brucellosis while the other lesions were considered to be related to reduced testosterone (atrophy of the prostatic acini) possibly caused by stress or by toxicants. Another high dose dog had moderate diffuse tubular degeneration/depletion of the testes, decreased sperm in the epididymis and an increase in the numbers of sloughed germ cells/cell debris in the epididymis. The prostatic acini of this animal showed normal secretory activity, indicating normal testosterone levels. This finding is considered potentially test article related.

It is the view of the DS that although there were single indications for adverse effects in several studies, no consistent pattern could be observed, nor could the findings be reproduced in the respective other studies. They also noted some methodological limitations in the studies. Overall, the DS sees "some evidence" but not "clear evidence" for adverse effects on fertility and therefore, proposes a classification in category 2 (Repr. 2; H361f).

Comments received during public consultation

One MSCA agreed with the classification proposal of the DS for category 2 for fertility.

Assessment and comparison with the classification criteria

Comparison with the criteria
Category 1A:
Known human reproductive toxicant.
No signs of reproductive disorder has been reported for alcoholic patients treated with cyanamide for up to a few years. There have not been any reports of functional disorder of the testes. Classification in category 1A is not therefore considered appropriate.

Category 1B:
Presumed human reproductive toxicant largely based on data from animal studies; clear evidence of an adverse effect on development 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.

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 and an anti-thyroid 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, i.e. effects on the testis in three dog studies. Consideration of species sensitivity with regard to thyroid hormone homeostasis indicates that the rat is particularily sensitive to anti-thyroid substances resulting in

hypothyroidism. There is some support for an intermediate sensitivity of dogs, in between rats and humans. The relationship between thyroid hormones and testicular development and function is recognised (Wagner *et al*., 2008) and provide support for classification in category 2 as it is plausible to conclude that the anti-fertility effect is related to antithyroid hormone homeostasis for which a significant species difference in sensitivity exists.

The data are hence considered by the RAC to support classification in category 2 (Repr. 2, H361f).

RAC evaluation of reproductive toxicity: Developmental toxicity

Summary of the Dossier submitter's proposal

The DS proposed to classify cyanamide for developmental toxicity as Repr. 2 (H361d) on the basis of evidence of embryotoxicity and malformation seen in the rat (marginal in the rabbit) in the presence of significant maternal toxicity. In addition, the DS conclusion was supported by a new developmental toxicity study presented during the public consultation (Pique, 2014; see summary below).

1. Developmental toxicity in Crl:CD SD rats (Morseth, 1989):

Aqueous hydrogen cyanamide was administered by gavage from GD days 6-15 in a GLP, guideline compliant study considered acceptable by the DS. Doses of 0, 5, 15 and 45 mg/kg bw/d of pure cyanamide active substance were given to 25 pregnant females/dose group.

Significant maternal toxicity was seen from 15 mg/kg bw/d. There were no deaths, but hypoactivity was seen in 8 dams of each dose group, during the first two exposure days, and the corrected body weight gain was reduced compared to the control. Mean food consumption was reduced by 8-11 % in the mid dose group and by 23-24 % in the high dose group during gestation days (GD) 6-20. The finding of maternal toxicity at these dose levels is consistent with the observations from sub-chronic toxicity studies in the rat. The table below presents the details of maternal effects and litter data.

Maternal and litter data

Dose level (mg/kg bw/d)	0	5	15	45
No of females in study	25	25	25	25
Females with hypoactivity	0	0	8	8
Pregnant dams	25	25	25	25
Dams with total litter loss	0	0	0	1
Body weight gain day 6 - 16 (g)	50.2	40.9*	31.9*	6.2*
Body weight gain day 6 - 20 (g)	105.8	94.2*	81.4*	51.0*
Food consumption day 6 - 16 (% of control)	100	96	89*	76*
Food consumption day 16 - 20 (% of control)	100	96	92*	77*
Carcass weight (g)	287.0	280.6	265.2*	243.9*
Net weight change from day 0 (g)	53.1	47.8	37.9*	15.5*
Gravid uterus weight (g)	77.5	75.3	69.0	61.4*
Corpora lutea (mean)	17.4	17.3	16.9	16.9
Implantation sites (mean)	14.7	14.5	13.9	14.2
Live litter size (mean)	14.2	14.1	13.3	13.8
Viable foetuses (%)	100	100	100	96
Dead foetuses (%)	0	0	0	4
Early resorptions (total)	12	9	11	26

Dose level (mg/kg bw/d)	0	5	15	45
Late resorptions (total)	1	0	3	0
Post implantation loss (mean %)	3.6	2.3	3.7	7.3

*statistically significant ($p<0.05$)

Corpora lutea, implantation number, litter size and viability were not affected. A possible increase in early resorptions was not statistically significant, but was not compared to HC data to confirm a lack of relationship with treatment (12, 9, 11, 26 early resorptions at 0, 5, 15 and 45 mg/kg bw/d, respectively). Mean foetal weights were significantly reduced ($p<0.05$) at 45 mg/kg. At this dose level there was a clear increase in diaphragmatic hernia (5(7) compared to 0(0) in controls (Table 141, p. 182 of the CLH report). In addition, skeletal malformations, mainly of the vertebrae, were noted in a few foetuses of this group. Variations (reduced ossification, possible interference with the process of rib ossification) were present to a greater extent in the high dose group and corresponded to the reduction in foetal weight. These variations included unossified hyoid body, incomplete ossification of the skull, bipartite vertebral centra, incomplete ossification of vertebral arches, less than four caudal vertebrae ossified, unossified sternebrae, incomplete ossification of the sternebrae, 14th rudimentary ribs, wavy or bent ribs and unossified pubes.

A mechanism of action for the diaphragmatic hernia was proposed which relates to a possible inhibition of aldehyde dehydrogenase (Raldh2), a major retinoic synthesising enzyme. The sequence of events was described as follows:

1) Cyanamide enters the maternal blood circulation and is metabolised to the active metabolite nitroxyl (HNO).
2) HNO enters the fetal blood circulation and reaches the tissue of the developing diaphragm.
3) HNO inhibits retinaldehyde dehydrogenase genes (Raldh2/ALDH1A2) of the diaphragmatic tissue, thereby reducing the concentration of retinoic acid in the tissue.
4) Decrease of retinoic acid during the retinoic acid-dependent diaphragm formation disrupts normal tissue development and leads to diaphragmatic hernias.
Metabolic activity leading to malformations (e.g. conversion of cyanamide to nitroxyl postulated to be responsible for the aldehyde dehydrogenase (ALDH)-inhibitory properties of cyanamide) significantly takes place in the maternal liver. The foetal liver does not have sufficient metabolic capacity to bioactivate cyanamide. Thus, a specific maternally-mediated mechanism leading to malformations is postulated.

The DS reported that nitrofen and three other teratogens which induce diaphragma defects in rodents have been shown to inhibit Raldh2 *in vitro* with a good correlation between teratogenic potency and inhibitory activity (Mey *et al*., 2003). Based on this, for cyanamide it was proposed to follow the same mode of action. This mechanism was not accepted by the PPP notifier as the chemicals (including nitrofen) acting through this mechanism cause multiple malformations including hernias. The mode of action was considered plausible by the DS but not specifically proven. A new study (Pique, 2014) was submitted during the PC which further explores the question of cyanamide induced diaphragmatic hernias.

2. Developmental toxicity in SD rats (Pique, 2014):

In this study, hydrogen cyanamide was administered by gavage at dose levels of 3, 15 and 45 mg/kg bw/d (active substance) to groups of 25 mated female SD rats from GD 6 to 19. Treatment-related clinical signs were restricted to the 45 mg/kg bw/d group. There was a dose-related and statistically significant reduction in overall mean body weight gain and food consumption during the dosing period in the 15 and 45 mg/kg bw/d 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 (GD 6 to GD 12). The effect was confirmed by lower mean net body weight change (i.e. maternal body weight change from GD 6 to GD 20 minus gravid uterus weight) in the 15 and 45 mg/kg bw/d groups (27 g and -34 g, respectively) compared with the control (48 g). The table below presents the details of maternal effects and litter data.

Summary of maternal and litter data.

Dose level (mg/kg bw/d)	**0**	**5**	**15**	**45**
No of females in study	25	25	25	25
Pregnant dams	25	24	25	23
Corpora lutea (mean)	15.2	14.7	14.6	14.6
Implantation sites (mean)	14.1±2.0	14.0±2.5	14.0±2.0	14.1±1.7
Early resorptions (%)	6.96±8.18	8.63±9.98	10.23±7.25	8.06±8.07
Late resorptions (mean%±SD)	0.87±2.42	3.40±12.30	0.77±2.87	3.14±8.39
Pre-implantation loss (mean%±SD)	6.51±9.30	5.36±8.75	3.72±7.48	3.2±4.78
Post implantation loss (mean %)	7.83±9.27	12.03±14.13	11.00±7.88	11.20±13.27
Body weight gain day 6 - 16 (g)	50.2	40.9*	31.9*	6.2*
Body weight gain day 6 - 20 (g)	105.8	94.2*	81.4*	51.0*
Food consumption day 6 - 16 (% of control)	100	96	89*	76*
Food consumption day 16 - 20 (% of control)	100	96	92*	77*
Carcass weight (adjusted) (g)	315.7	315.2	300.7*	236.6*
Net weight change (adjusted) from day GD6-20 (g)	47.75±10.67	43.7610.59	27.15±11.24**	-34.34±20.30***
Gravid uterus weight (g)	80.18	75.48	69.80*	51.39***
Live litter size (mean)	13±2.4	12.5±2.9	12.5±2.2	12.6±2.7
Mean foetal weight (G)	4.01±0.21	3.97±0.30	3.67±0.31**	2.47±0.35***
Dead foetuses (%)	0	0	0	0
% male foetuses	47.49	50.97	50.14	44.70

*/**statistically significant (p-values not indicated)

There was clear evidence of general developmental toxicity (delay in development) in the 45 mg/kg bw/d 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 included 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. These effects could not be clearly attributed to the observed maternal toxicity by the author (Pique, 2014). The table below presents details of relevant foetal observations.

Summary of relevant foetal observations

Dose level (mg/kg bw/d)	0	3	15	45
Foetal abnormalities: litter incidence (foetal incidence)				
Diaphragma hernia	0	0	0	10 (7)**
Testis malpositioned	0	0	0	7(7)**
Great blood vessel defects -aortic arch narrowed -multiple abnormalities	0 0 0	0 0 0	1(1) 0 1(1)	4(4) 1(1) 3(3)
Fused sternebrae	0	0	0	2(1)
Hemisterebrae	0	0	0	3 (3)
Wavy/bent ribs	0	0	0	47 (19)**
Incomplete ossification -skull -limbs -sternebrae -ribs -vertebrae -pervic girdle	- - - - - - -	- - - - - - -	- - - - - - -	↑↑↑** ↑↑↑** ↑↑↑** ↑↑↑** ↑↑↑** ↑↑↑** ↑↑↑**
Thickened ribs	0	0	0	10(16)*
Short rib	0	0	0	4(4)
Thoracic Vertebra: bipartite ossification of centrum	1 (1)	1 (1)	2 (2)	14 (10)**
Hemicentrum	0	0	0	2 (1)**

*/**statistically significant (p-values not indicated)

The results of this new study confirm those of the study of Morseth (1989). In both studies visceral and skeletal malformations associated with marked maternal toxicity were observed at the high dose (45 mg/kg bw/d) and clearly support the proposal of the DS for classification for developmental toxicity.

3. Developmental toxicity in the NZW rabbit (Koeter, 1989):

In rabbits dosed with 0, 2, 6 and 18 mg/kg bw/d cyanamide, maternal toxicity was noted in the high dose group. Body weight loss and a decrease in body weight gain during the period of substance application was observed. One female had a total litter resorption. A reduction in gravid uterine weight was noted as a consequence of lower live litter sizes and marginally reduced foetal weights. On the foetal level this prenatal developmental toxicity in the high dose group presented as embryo- and foetolethality and a higher prevalence of small foetuses. A number of soft tissue changes have been reported. Among these findings, minor eye anomalies (retina folds) were observed in all groups including the control group. A dose-related increase in severity was suggested by the distribution of unilateral and bilateral observations. However, a recently published study comparing the retinal structure of control rabbit foetuses by magnetic resonance imaging of the fresh specimen with their state after Bouin's fixation provides evidence that slight retinal folds are rather a processing artefact than a treatment-related effect (French *et al.*, 2008). Taking this new information into account, no indication for teratogenicity was detected in rabbits up to and including the high dose level.

In conclusion, an increased incidence of diaphragmatic hernia was seen in two acceptable rat developmental toxicity studies, using comparable regimes. There were additional low incidences of other skeletal malformations at this dose in the Morseth (1989) study. In addition, a possibly treatment-related increase in defects of the great blood vessels (4 (4) foetuses (litters)) could not be clearly attributed to the observed maternal toxicity by the author in the recent study of Pique (2014).

Clear evidence of general embryotoxicity was seen at the high dose in both studies.

Increased post-implantation loss and numbers of small foetuses was seen in conjunction with significant maternal toxicity in the rabbit. All developmental toxicity including specific malformations were seen in conjunction with severe maternal toxicity in the rat. In addition, significant embryotoxicity was seen in the multigeneration studies reviewed above which supports the developmental toxicity classification proposal.

Comments received during public consultation

A new developmental toxicity study (Pique, 2014) was submitted by the notifier during the PC, addressing the observation of increased incidence of diaphragmatic hernia seen in the Morseth (1989), study (described above).
The classification proposal of the DS as Repr. 2; H361d was supported by one MSCA.

Assessment and comparison with the classification criteria

Category 1A:
Known human reproductive toxicant.
No signs of reproductive disorder or developmental toxicity has been reported for alcoholic patients treated with cyanamide for up to a few years. Classification in category 1A is not therefore considered appropriate.

Category 1B:
Presumed human reproductive toxicant
Classification in this category is based on "...*clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects."*

An increased incidence of diaphragmatic hernia was seen in two acceptable rat developmental toxicity studies, using comparable treatment regimen. There were additional low incidences of other skeletal malformations at this dose in the Morseth (1989) study. In addition a possibly treatment-related increase in defects of the great blood vessels (4 (4) foetuses (litters)) could not be clearly attributed to the observed maternal toxicity by the author in the recent study of Pique (2014).
Clear evidence of general embryotoxicity was seen at the high dose in both studies.
Increased post-implantation loss and numbers of small foetuses was seen in conjunction with significant maternal toxicity in the rabbit. All developmental toxicity including specific malformations were seen in conjunction with severe maternal toxicity in the rat.

The mechanism of action proposed relates to the inhibition of Raldh2 and its possible relevance to humans. While general maternal toxicity was clearly present, the malformations were attributed, according to the DS, to a separate specific mechanism. RAC has doubts concerning the relevance of the proposed mechanism to the effects observed and which is not specifically proven for cyanamide. However, general maternal toxicity is recognised to contribute to the multiple embryofoetal effects also seen. RAC found it difficult to conclude that the significant maternal toxicity occurring in conjunction with most of the adverse effects on the embryo/foetus did not contribute to the outcome. Therefore classification in category 1B may not be appropriate.

Category 2:
Suspected human reproductive toxicant
According to the criteria, where other (maternal) toxic effects are marked (and/or the quality of the evidence is not sufficiently convincing for category 1, category 2 should be considered. 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 RAC considers the criteria for 'clear evidence' of a significant adverse effect on the embryofoetus to be fulfilled, while recognising significant maternal toxicity which occurred in conjunction with most of the adverse effects on the embryo/foetus. RAC concludes therefore that the data support classification of cyanamide as Repr. 2 (H361d) rather than Cat 1B.

ENVIRONMENTAL HAZARD ASSESSMENT

RAC evaluation of environmental hazards

Summary of the Dossier submitter's proposal

Cyanamide does not currently have a harmonised classification for environmental hazards. The DS outlined only the most important aquatic studies considered relevant to classification. The DS proposed to classify cyanamide as Aquatic Chronic 1: H410, M=1.

Degradation

The water solubility of cyanamide is >560 g/L at 20°C (pH 7). At 25°C no hydrolytic degradation was observed so the test was performed at elevated temperatures. Several calculation methods were applied, and the results of these calculations showed that cyanamide is hydrolytically stable within the pH range of 5 to 9 (Tables 153 and 154, CLH report). Cyanamide is photolytically degraded in aqueous solutions at 25°C. Photolytic DT_{50} values were determined to be 28.9 d and 38.5 d in the light exposed samples at pH 5 and pH 7, respectively. DT_{50} values of the dark control samples were calculated to be 116 d and 139 d at pH 5 and pH 7, respectively. In summary, light exposed samples of cyanamide degraded about four times faster than dark control samples (Table 155, CLH report). According to table 156 of the CLH report, cyanamide is stable in the atmosphere, it does not react with OH^- radicals based on the Atkinson calculation (Peter, 2003). However, the DS (and the original RMS for the draft assessment report; DAR) was not convinced of the adequacy of this method in determining photochemical degradation in air. Furthermore, studies have been performed on the phototransformation potential of cyanamide in soil. However, these studies were neither presented nor considered for the overall assessment of the stability of cyanamide in the CLH report (see the section 'Additional Key Elements', Burri, 2000, in the Background Document) where the photolytic half-life of cyanamide on soil surfaces was 1.45 days). In addition, the low persistence in a laboratory soil was confirmed by a DT_{50} field value of 1.26 days, determined in a study on aerobic soil metabolism (Schmidt, 1990) and of 4.3 days in surface water (Völkl, 2000).

The biodegradability of cyanamide was investigated in a primary study on ready biodegradability according to OECD TG 301 E (van der Hoek & Hanstveit, 1988). In this test, cyanamide was shown to be not readily biodegradable with 0% degradation within 28 days. The biodegradability of sodium acetate was used as a measure for the microbial activity of the inoculum, and to detect any inhibition of this activity due to the presence of high concentrations of cyanamide. Sodium acetate was completely degraded within 7 days and the presence of cyanamide did not affect this process.

A second study on ready biodegradability by measuring the CO_2 evolution was performed but could not be validated due to considerable deviations from OECD TG 301 B concerning the concentration of cyanamide applied. Details taken from the original DAR show that the Matla & Hanstveit (1990) study was performed in order to determine the influence of limiting factors (i.e. nitrogen source, test substance and inoculum concentrations) on the biodegradability of cyanamide. In this study, when cyanamide served as the only nitrogen source (and sodium acetate as the carbon source), degradation rates up to 100% were obtained at day 13. When cyanamide served as the sole nitrogen and carbon source, very little degradation took place (about 22 % within 8 weeks). These results also preclude microbial inhibition in the presence of cyanamide as a reason for the lack of degradation in the original ready biodegradability test. The authors speculated that the presence of another nitrogen source prevents cyanamide from being degraded by microbial action. Considerable deviations from the OECD TG 301 guidelines precluded the DS from considering the study as acceptable from a regulatory point of view and incorporating it into the CLH report (though it was considered acceptable in the DAR). In addition, in a water sedimentation study by Völkl (2000) which was not taken into consideration in the CLH report, rapid degradation of cyanamide was clearly demonstrated under environmentally realistic conditions in two aerobic water/sediment model systems (see section iii in 'Additional Key Elements', Burri, 2000, in the Background Document). In both systems cyanamide was degraded by 99.9 % within 28 days. Greater than 83 % was shown to be fully mineralised to CO_2 within 28 days either directly or via the intermediate metabolite urea.

Bioaccumulation

The log Pow is -0.72 at 20°C (pH ~ 6.8). An approximate estimation of the bioconcentration factor BCF_{fish} on basis of log Kow = -0.72 was performed using the standard equation (74) given in the EU Technical Guidance Document (TGD) on Risk Assessment (2003), Part II, 3.8.3.2. The estimated BCF_{fish} = 0.049 (on a wet weight basis). Based on this low estimate, an experimental study with fish was not required. No other indicators point to an intrinsic potential for bioconcentration; the surface tension, for instance, is 72.86 mN/m and thus lies above the trigger value of ≤ 50 mN/m. The DS concluded that with the log Pow substantially less than the cut off value of ≥ 4, and an estimated BCF value substantially less than the cut off value of ≥ 500, cyanamide is not bioaccumulative.

Aquatic Toxicity

Acute toxicity

The CLH report presents one acute toxicity study for fish, one for Daphnia, one for algae and one for cyanobacteria. The lowest values are presented in the table below, with the most relevant study for classification outlined in bold.

Lowest acute aquatic toxicity data available

Species	**Test guideline**	**Test type, duration, reference**	**Result**
Lepomis macrochirus (Bluegill Sunfish)	EPA 660/3-75-009 (1975) equivalent to OECD 203, GLP	96 h, static McAllister, W.A. *et al*. (1985) Doc. No. 821-002	LC_{50} 43.1 mg/L
Daphnia magna	**OECD 202 (1981), no GLP**	**48 h, static Adema, M.M. (1983) Doc. No. 822-001**	**EC_{50} 3.2 mg/L**
Pseudokirchneriella subcapitata	OECD 201 (1984), GLP	72 h, static Seyfried, B. (2000), Doc. No. 823-003	ErC_{50} 14.7 mg/L

Anabaena flos-aquae	OECD 201 (1984), GLP Judged invalid by the DS.	72 h, static Hertl, J. (2000), Doc. No. 823-004	[1]ErC_{50} 0.65 mg/L

[1] considered invalid

The growth inhibition test (Hertl, 2000) with *Anabaena flos-aquae* was considered by the DS to be unvalid. 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 did not grow exponentially. According to OECD TG 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 were 2.6 between day 0-1, 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 was 114%. Consequently this test cannot be used for the effect assessment for cyanamide. As there is another, valid algal growth inhibition test available (*Pseudokirchneriella subcapitata*; Seyfried, 2000) for cyanamide, a repeat of the study was not required.

There was another growth inhibition test with *Anabaena flos-aquae* reported in the DAR (Wenzel, 2003) using a different cyanamide product formulation. However, the original RMS reported the test as non-valid because the replication factor of 4.3 in the control cultures was regarded as too small. In addition there was no cell proliferation in the control cultures between 24 and 48h and a continuous exponential growth could not be maintained.

Chronic toxicity

There was adequate chronic toxicity data available for two trophic levels only. There is one chronic toxicity study for fish, and one for *Daphnia* reported in the CLH report. The lowest values are presented in the table below, with the most relevant study for classification outlined in bold.

Lowest chronic aquatic toxicity data available

Species	**Test guideline**	**Test type and duration**	**Result**
Oncorhynchus mykiss	OECD 204, U.S. EPA-FIFRA, Guideline 72-1, GLP	21-d, flow-through Bowman & Herzig, (1990), Doc. No. 826-001	NOEC 3.7 mg/L
Daphnia magna	**US EPA–FIFRA 72-4 (b) guideline, GLP**	**21-d, flow-through Murrell & Leak (1995), Doc. No. 827-002**	**NOEC reproduction 0.104 mg/L**

The lowest acute toxicity value for cyanamide is a 48 hour EC_{50} of 3.2 mg/L. The lowest reliable chronic toxicity value for cyanamide is a 21-day NOEC of 0.104 mg/L. There was no chronic algae data but by using the surrogate approach to the acute algae data (ErC_{50} 14.7 mg/L) it can be seen that it does not result in a stricter classification than the chronic *Daphnia* data (i.e. using the results from the acute algae study, the criteria are satisfied for Aquatic Chronic 3; ErC_{50} > 10 to ≤ 100 mg/L).

The DS proposed to classify cyanamide as Aquatic Chronic 1: H410, M=1.

Comments received during public consultation

One MSCA agreed with the original classification proposal by the DS. Two MSCA queried why the water sedimentation study by Völkl (2000) was not taken into consideration in the CLH report and one of these MSCAs wanted more detail with regards to the invalidity of the Hertl (2000) study with *Anabaena flos-aquae*. Industry submitted position papers that gave corrections to a number

of toxicity results that impact on classification, supporting an alternative classification of Aquatic Chronic 3 – H412.

The DS took note of the corrections requested during PC and supported that classification of cyanamide be amended accordingly by RAC. The DS concluded in the RCOM (in contrast to statements in the CLH report) that when evaluating all of the evidence presented, cyanamide may be considered rapidly degradable.

Phototransformation in soil
A summary of the key data on phototransformation potential of cyanamide in soil is presented in the original DAR below (Burri, 2000).

DT_{50} and DT_{90} values of cyanamide in irradiated and non-irradiated soil samples

	DT_{50}	**DT_{90}**	**Correlation coefficient**	**Kinetic**
	[days]			
Irradiated	1.45	4.85	0.9904	first-order
Non-irradiated	4.22	14.03	0.9899	first-order

The original RMS concluded that cyanamide is rapidly degraded on the soil surface with a photolytic half-life of 1.45 days. A relatively fast degradation with a DT_{50} value of 4.22 days could also be observed in the dark control samples. Whilst in the non-irradiated samples cyanamide is mainly degraded to CO_2, indicating a complete mineralisation, the photolytic degradation leads to the degradation products urea and dicyandiamide.

Low persistence in a laboratory soil
A summary of the study on aerobic soil metabolism of cyanamide is presented from the original Plant Protection DAR below (Schmidt, 1990; Doc No 722-001)

Findings
Material balance: The total recovery ranged from 97.8% to 108% of initial measured dose (IMD) with a mean recovery of 101% of IMD. Extractable residues decreased from 96.7% of IMD immediately after application to 0.37% at day 14. Unextractable (bound) residues increased from 3.39% of IMD at day 0 to 5.64% at day 14. Cumulative volatile residues increased to 97.1% of IMD at day 14. The majority (94.6% of IMD) was identified as [$^{14}CO_2$] by precipitation with $BaCl_2$. Radioactivity observed in the H_3PO_4 and ethylene glycol traps accounted for <0.1% and 2.63% of IMD, respectively.

Principal degradation products: TLC analysis of the soil extracts revealed the presence of cyanamide and one degradation product, which was identified as dicyandiamide. Cyanamide decreased from 92.5% of IMD at day 0 to 0.051% at 14 days. Dicyandiamide decreased from 0.431% at day 0 to 0.032% at day 14.

DT_{50} and DT_{90} values: The degradation of cyanamide in a sandy loam soil was best described by first order degradation kinetics. The DT_{50} value was calculated to be 1.26 days. The DT_{90} value was not calculated within the report, but the data given in the report was taken to calculate the DT_{90} during the dossier compilation using the model ModelManager. The DT_{90} value of cyanamide was calculated to be 1.94 days assuming first order degradation kinetics.

Conclusion:

In summary, the primary aerobic pathway by which the parent compound disappears from soil is the final degradation to [$^{14}CO_2$] (complete mineralisation). This fraction accounted for

approximately 94.6% of applied radioactivity after 14 days. The half-life of cyanamide was calculated using a first order kinetic model and was found to be 1.26 days. The calculated time to 90% degradation was 1.94 days.

Route and Rate of Degradation in Aerobic Aquatic Systems

According to the CLP, Annex I, section 4.1.2.9.2 "*Other evidence of rapid degradation in the environment may therefore also be considered and are of particular importance where the substances are inhibitory to microbial activity at the concentration levels used in standard testing. 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 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 %). A summary of the study is presented from the original DAR below (Völkl, 2000; Doc No 714-002).

Volatile degradation products: The formation of CO_2 was very high, accounting for a maximum of 86.1 % and 83.5 % of the applied radioactivity on day 28 for the river and pond systems, respectively. Other volatile compounds did not exceed 0.1 % of the applied radioactivity.

Cyanamide concentrations: The amount of [^{14}C]-cyanamide in the water phases decreased continuously to a minimum of 0.1% of applied radioactivity on days 12 and 28 in the river and pond systems, respectively. In the sediment extracts of the river system, cyanamide was detected from day 1 to day 6 at a maximum concentration of 3.1% of applied radioactivity. In pond sediment extracts cyanamide was detected from day 1 to day 12 amounting to a maximum of 4.7% of applied radioactivity.

Principal degradation products: In the river system, cyanamide degraded to 8 minor radioactive fractions. Two degradation products were identified as urea and dicyandiamide amounting to 6.7% and 0.3% of applied radioactivity, respectively. One degradation product, which accounted for 5.5% of applied radioactivity, could not be identified by chromatography using the available reference standards. All other degradation products present did not exceed a maximum level of 0.3% of applied radioactivity.

In the pond system, cyanamide was degraded to 4 components. Urea was the main degradation product amounting to a maximum of 13.4% of applied radioactivity on day 1 and decreasing continuously thereafter to represent 1.2% of the applied radioactivity on day 21. All other degradation products present did not exceed a maximum level of 5% of applied radioactivity.

DT_{50} and DT_{90} values of cyanamide: The degradation of cyanamide in water/sediment systems was best described by first order degradation kinetics. The DT_{50} values in the water phase were calculated to be 2.3 and 4.3 days for the river and pond systems, respectively, and the DT_{90} values were determined to be 7.7 days and 14.4 days, respectively. In the total water sediment systems cyanamide was degraded with half-lives of 2.5 days (river) and 4.8 days (pond). The DT_{90} values for the total systems were calculated to be 8.2 days in the river system and 15.8 days in the pond system.

DT_{50} and DT_{90} values of urea: The DT_{50} and DT_{90} values of urea were also calculated using first-order kinetics. The DT_{50} values in the water phase were calculated to be 2.7 and 7.5 days for

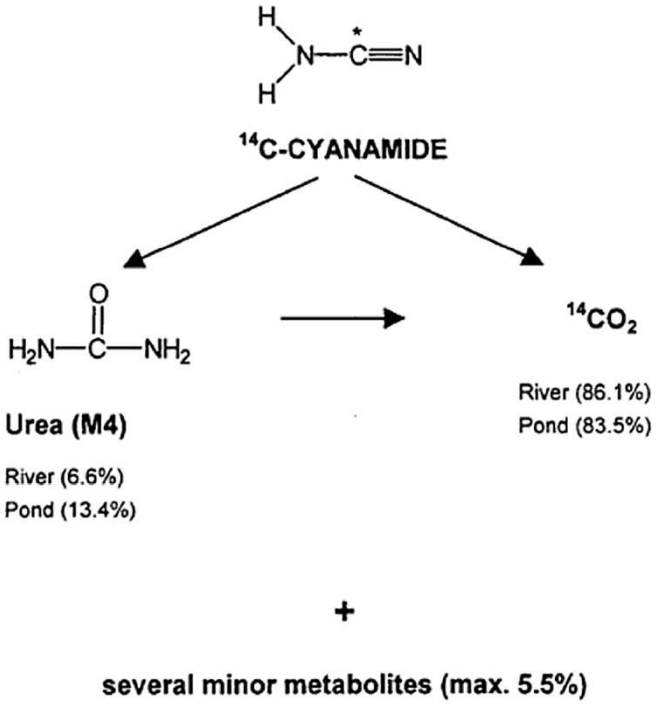
the river and pond systems, respectively, and the DT_{90} values were determined to be 9.1 days and 11.6 days, respectively. In the total water sediment systems urea was degraded with half-lives of 2.9 days (river) and 8.0 days (pond). The DT_{90} values for the total systems were calculated to be 9.6 days in the river system and 26.7 days in the pond system.

DT_{50} and DT_{90} values for cyanamide and urea in water/sediment systems are presented in the table below (from DAR).

DT_{50} and DT_{90} values for cyanamide and urea in water/sediment systems

	System	**DT_{50} (days)**	**DT_{90} (days)**	**r^2**
cyanamide	**River**			
	water total system	2.3 2.5	7.7 8.2	0.9969 0.9957
	Pond			
	water total system	4.3 4.8	14.4 15.8	0.9898 0.9895
Urea	**River**			
	water total system	2.7 2.9	9.1 9.6	0.966 0.965
	Pond			
	water total system	7.5 8.0	11.6 26.7	0.939 0.935

The proposed degradation pathways of cyanamide in aquatic systems are presented in the figure below (from the DAR).



Conclusion: The elimination of ^{14}C-cyanamide from the water/sediment systems proceeded mainly via mineralisation to CO_2. Degradation of cyanamide to other metabolites and incorporation into the organic matter of the sediment were of minor importance. The estimated half-life of cyanamide from the water phase of the aquatic systems was 2.3 days for the river system and 4.3 days for the pond system, respectively. One major metabolite was detected in the

pond system (13.4 % of applied radioactivity) and identified as urea. The estimated DT_{50} value of urea was 7.6 days in the total pond system indicating a transient nature. In the river system no metabolite exceeded the 10% level.

Assessment and comparison with the classification criteria

Degradation

RAC disagrees with the original DS proposal to consider cyanamide as not rapidly degradable. The substance is hydrolytically stable, but rapid degradation of cyanamide was demonstrated under environmentally realistic conditions in two aerobic water/sediment model systems. In both systems cyanamide was degraded by 99.9% within 28 days. Of the applied radioactively-labeled cyanamide > 83 % could be shown to be fully mineralised to CO_2 within 28 days either directly or via the intermediate metabolite urea. Cyanamide is therefore considered by RAC to be rapidly degradable according to the CLP criteria.

Bioaccumulation

RAC agrees that cyanamide has a low potential to bioaccumulate based on an estimated fish BCF value of 0.049.

Aquatic toxicity

There are adequate acute and chronic toxicity data available on fish, Daphnia, and algae. 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 $EC_{50} \leq 1$ mg /L was not triggered for cyanamide. The lowest acute value found was an EC_{50} of 3.2 mg pure cyanamide/L for *Daphnia magna*. Classification for acute aquatic toxicity is not required.

Chronic aquatic toxicity:
Cyanamide was found to be rapidly degradable. There was adequate chronic toxicity data available for two trophic levels only. There was no chronic algae data but using the surrogate system to acute algae data (72hr, ErC_{50} 14.7 mg/L), the criteria are satisfied for Aquatic Chronic 3; ErC_{50} > 10 to ≤ 100 mg/L and does not suggest stricter classification than with the chronic *Daphnia* data. The most stringent outcome in this case is identical to that for rapidly degradable substances using the lowest relevant chronic NOEC value. The chronic NOEC (YAD) for *Daphnia magna* was 0.104 mg pure cyanamide/L which is greater than the limit value of 0.1 mg/L for the classification of rapidly degradable substances as "Aquatic Chronic 2". Cyanamide may be classified as Aquatic chronic 3 – H412 "Harmful to aquatic life with long lasting effects."

Conclusion on classification

The RAC concludes that classification of cyanamide as Aquatic Chronic 3; H412 is warranted according to the CLP Regulation. The classification is based on the substance being rapidly degradable, non-bioaccumulative and harmful to aquatic organisms.

Additional references

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.

Capen, C., 1979. Mechanistic data and RA of selected toxic end points of the thyroid gland. Tox Path 25 (1): 39-48).

C&L Specialised Experts, 1999; ECBI/49/99 – Add. 1 Rev. 2

Daminet and Ferguson (2003). Influence of drugs on thyroid function in dogs. J Vet Intern Med; 17: 463-472.

Davidson B, Soodak M, Strout HV, Neary JT, Nakamura C, Maloof F (1979). Endocrinology. 1979 Apr;104(4):919-24. Thiourea and cyanamide as inhibitors of thyroid peroxidase: the role of iodide.

French, J. *et al*. (2008) Retinal folding in the term rabbit fetus - Developmental abnormality or fixation artifact? Reproductive Toxicology 26, 262-266.

Greaves (1990). Histopathology of preclinical toxicity studies: Interpretation and relevance in drug safety evaluation, Elsevier.

Haseman *et al*. (1998). Tox Path, vol 26 (3); 428-441

Wagner M.S., Wajner S.M., and Maia A.L. (2008) The Role of Thyroid Hormone in Testicular Development and Function. J Endocrinol. 2008, Dec; 199(3): 351–365.

Nickel, G. (2014) 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"

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

Wenzel, A. (2003): Alga, growth inhibition test - Effect of Alzodef on the growth of cyanobacteria Anabaena flos-aquae. Fraunhofer Institute for Molecular Biology and Applied Ecology, Schmallenberg, Germany. GLP, unpublished, Doc. No. 823-006

Woutersen, R.A.; Bruijntjes, J.P. (2005): Historical data in dogs on atrophy and/or reduced spermatogenesis in dogs.

ANNEXES:

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

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