Developmental effects and maternal toxicity of Hydrogen cyanamide

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

The mid and high dose levels of the second study (Pique, 2014) were therefore selected solely to replicate the design of a previous prenatal developmental toxicity study in the Sprague-Dawley rat (Morseth, 1989) which had yielded equivocal results according to the assessment of Harris (2012). In the older study a moderate maternal toxicity in the mid dose and a marked maternal toxicity in the high dose were elicited. In the newer study these effects were even more pronounced particularly in the high dose group. Therefore, based on the principles of reproductive toxicology and today's knowledge regarding the toxicological properties of HC, the high dose for a developmental toxicity study would have been selected in the range of 15 mg/kg bw/day as this dose clearly meets the validity criteria of OECD Guideline 414 for a high dose setting.

The relevant OECD Guideline 414 (Prenatal Developmental Toxicity Study, 2001) states that *"the highest dose should be chosen with the aim to induce some developmental and/or maternal toxicity (clinical signs or a decrease in body weight) but not death or severe suffering."* While here the definition of maternal toxicity is relatively vague, the closely related and newer OECD Guideline 426 (Developmental Neurotoxicity Study, 2007) is more precise, stating that *"the highest dose level should be chosen with the aim to induce some maternal toxicity (e.g., clinical signs, decreased body weight gain (not more than 10%) and/or evidence of dose-limiting toxicity in a target organ)".* Based on this, a body weight gain decrease of up to 10% would be desirable, but a loss in body weight is certainly not acceptable.

To address this issue the mean net maternal body weight change, i.e. carcass weight on GD 20 minus body weight on GD 6 is considered as the most relevant parameter for maternal body weight effects being a reliable indicator of maternal toxicity during the application period (Moore et al., 2013). The carcass weight is the terminal body weight minus uterine weight. The most relevant parameters are given in the following tables in bold letters. The respective

absolute and relative net body weight changes (relative to the control group) for both developmental toxicity studies are listed in the last two lines in bold letters in the tables below. For an easier comprehension of the maternal toxicity other relevant parameters like body weight on GD 20, body weight change GD 6-20, food consumption GD 6-20 and gravid uterus weight are also given.

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

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

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

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

Dose level (mg/kg bw/d)	**0**	**3**	**15**	**45**
Carcass weight (g)	**315.7**	**315.2**	**300.7***	**236.6***
Body weight GD 6 (g)	**268.0**	**271.4**	**273.5**	**270.9**
Net absolute body weight change GD 6-20 in grams (% of control in brackets)	**47.8**	**43.8 (-8)**	**27.1* (-43)**	**-34.3* (-172)**
Body weight on GD 20 (g)	395.9	390.7	370.5*	288.0*
Nody weight change GD 6-20 (g)	133.7	125.4	102.7*	21.7*
Food consumption GD 6-20 (g/day)	27.8	27.5	25.2*	14.9*
Gravid uterus weight (g)	80.2	75.5	69.8*	51.4*

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

GD = gestation day
* statistically significant

Applying the criteria of the Developmental Neurotoxicity Guideline 426 (2007) for a decreased body weight gain of (not more than) 10% in the high dose group to the study of

Morseth (1989) would have resulted in a net **maternal body weight increase of 25.4 g** and **not to a loss of 10.4 g (-137%)** in the high dose group.

On the other hand the net body weight gain of the mid dose animals of 12.4 g (about -56% compared to the control) comes closer to the requirement of the new OECD Guideline 426 although still exceeding the proposed limit on body weight effects of about 10 % for a high dose group.

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

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

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

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

In contrast, the effects noted for the mid dose groups in both studies came closer to the guideline requirements for a high dose group, although still somewhat exceeding the recommendation as described in the Developmental Neurotoxicity Guideline 426 (2007).

Consequently, evaluation of the potential developmental effects of HC should be based on the findings in the mid dose group in both studies as this dose of 15 mg/kg bw/d already represents a reasonable worst case scenario with regard to the observed maternal toxicity.

Conclusion, proposal for classification

The findings in the high dose groups of both studies must be seen in conjunction with the severe maternal toxicity leading to massive reductions of net body weight gain in both studies associated with an extremely compromised health status of some animals in one of these studies. Due to the severe maternal toxicity the findings observed at 45 mg/kg bw/d should not be considered for developmental toxicity classification. The observations at the mid dose level (15 mg/kg bw/d), where still clear maternal toxicity was observed would, per se not warrant a classification for developmental toxicity.

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

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

References

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