Rationale for TEL, WES and NOAEC values for ethanedinitrile , Draft 1

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A) Summary of relevant chronic studies

1) **Ethanedinitrile:** In Lewis et al. 1984 study, they exposed animals, rats and monkeys to 25 ppm ethanedinitrile (12.5 ppm cyanide) and 11 ppm ethanedinitrile (5.5 ppm cyanide) for 6 hours/day, 5 days/week, for 6 months. No haematological, musculoskeletal, cardiovascular effects histopathological changes in e.g. kidneys, liver, thyroid, spleen, heart, lungs, bone marrow, cerebellum, cerebrum or changes in T3 and T4 were found at the higher concentration of 25 ppm.

   In this trial, a decreased body weight but no respiratory effects were reported in rats exposed to 25 ppm ethanedinitrile (12.5 ppm cyanide). Monkeys in the same trial exposed to 11 ppm ethanedinitrile (5.5 ppm cyanide) showed a decrease in total lung moisture content. Neither of these effects was considered to be an "adverse effect" by the rapporteur member state of EU in its recent assessment of Lewis et al. 1984 study. In addition, monkeys demonstrated transitory behavioural changes exposed to 25 ppm ethanedinitrile (12.5 ppm cyanide) at the beginning of the exposure period but this disappeared with time.

   In conclusion, it is considered that no adverse effects were found in monkeys at 11 ppm and 25 ppm ethanedinitrile (5.5 ppm cyanide) exposure (Lewis et al. 1984). Since, Lewis 1984's assessment showed exposure of rhesus monkeys to 25 ppm of ethanedinitrile had no adverse effect, this concentration level was used to set a NOAEC in the EU registration of HCN as a biocide. This corresponds to a daily dose of ≥4.7 mg CN /kg bw (≥9.4 mg C2N2 /kg bw).

   Monkeys are considered to be an ideal model animal to study human toxicology as they have been shown to be more sensitive to cyanides than humans (NCR 2001). Therefore, the outcome from the monkey study can be regarded – for greater toxicological relevance than rat studies.

   It is worth noting however that rats are considered to be an even more sensitive to cyanides than monkeys and humans (NCR 2001). Data from this trial resulted in a NOAEC for rats of 25 ppm (EU HCN dossier). This corresponds to a daily dose of 5.2 mg CN /kg bw (10.4 mg C2N2 /kg bw).

Table 6.2.5 Ethanedinitrile -long term toxicity

Test	Tested organism	Concentration Dose	Result	Report References
Ethanedinitrile 180 day-inhalation study	Rats Monkeys	11 or 25 ppm 6h/d 5 d/w	NOAEL ≥ 25ppm C2N2 corresponding to daily doses ≥4.7 mg and 5.2 mg CN /kg bw in monkeys and rats, respectively.	Lewis 1984

2) **Cyanides** Chronic oral exposure with HCN together with sub chronic oral administration of inorganic cyanides in the drinking water or diet was conducted on albino rats (Howard, Hanzal 1955) . The results of the long term cyanide studies are summarised in table 6.2.6.

Another NTP study (NTP 1996) involving combined chronic inhalation toxicity (carcinogenicity study) of acetonitrile using rats and mice -is provided as a substitute in table 6.2.6. In this study, rats and mice exposed for 2 years to acetonitrile concentrations for up to 670 (rats) and 335 (mice) mg/m3 for 6h/d, 5d/w and there was no significant evidence of exposure-related clinical effects or non-neoplastic lesions were found..

Based on both studies, it is considered that prolonged exposure to cyanides will not modify or increase the cyanides toxic effects. As a consequence no special chronic (non-carcinogenic) toxicity studies in laboratory animals are not warranted.

Table 6.2.6 Cyanide _long term toxicity

Test	**Tested organism**	**Concentration Dose**	**Result**	**Report References**
Acetonitrile 2 year-inhalation study	F344/N rats and $B6C3F_1$ mice	100 – 400 ppm 50 – 200 ppm	No non-neoplastic effects at exposure corresponding to daily doses ≥ 10 mg cyanide per kg bw (top dose)	NTP 1996[1]
HCN 2 year dietary study	Albino rats	100 or 300 ppm HCN in diet	No effects at daily doses about 10 mg/kg bw. (top dose)	Howard, Hanzal 1955[2]

3) **In conclusion** Three chronic studies are described in detail in the EU toxicological dossier for ethanedinitrile.

It is noted that Lewis et al. (1984) exposed monkeys and rats to ethanedinitrile at daily doses of ≥4.7 mg and 5.2 mg CN /kg bw. This study is important since the exposure is directly related to ethanedinitrile. This chronic toxicity study conducted using ethandinitrile can be compared with other chronic studies using acetonitrile (NTP 1996) and hydrogen cyanide (Howard, Hanzal 1955). It should be noted that the later study (Howard Hanzel 1955) exposed animals to twice the daily dose with no significant adverse effects.

[1] Toxicology and Carcinogenesis Studies of Acetonitrile In F344/N Rats And B6C3F Mice (Inhalation Studies); NTP, Toxicology Report Series No. 447. (NIH Publication 96-3363)
EU dossier file name: 07_EDN_NTP_Acetonitrile_1996_MARK.pdf

2 John W. Howard and R. F. Hanzal, Chronic Toxicity for Rats of Food Treated with Hydrogen Cyanide;(1995) Hazleton Laboratories, Falls Church, Va., Agricultural and Food Chemistry, Volume 3, April 1955, No.4
EU dossier file name: 07_HCN_HOWARD_chronic tox_1995_MARK.pdf

B) WES, TLV values

To mitigate any effects on workers and bystanders from continuous exposure to ethanedinitrile for 8 hours, 5 days a week the safe concentration which ensures no adverse effects needs to be established.

For ethanedinitrile this value is 10 ppm (WES-TWA)

- as identified by Worksafe New Zealand,
- as proposed for the EU, and,
- is legally binding in USA.

A TLV-TWA of 10 ppm EDN was set by the ACGIH from 1967 to 2016 (ACGIH 2016). However, in 2015 the ACGIH proposed a decreased value to 5ppm TLV-Ceiling. This resulted in the value being formally changed to 5ppm in 2016 (ACGIH 2016).

The change by the ACGIH was not based on any new data but was in fact based on data from the same endpoint study that had been used to calculate previous value of 10 ppm TLV-TWA (ACGIH 2001, 2015, 2016). This published study reported irritation in humans (McNerney,1960).

Draslovka considers that information provided in the McNerney (1960) report on the degree of irritation caused by exposure to ethanedinitrile is insufficient to be classified as an irritation hazard according to the CLP, GHS and respective OECD standards for irritation testing. On this basis Draslovka considers ethanedinitrile should not be considered a hazard and the McNerney report is not a sound basis for decreasing the TLV levels. In this area Draslovka disagrees with the changes made by the ACGIH.

To support its stance, Draslovka emphasis that there is no report of irritation or eye lacrimation in workers functioning in an environment where the TLV – TWA of 10 ppm has been used for 49 years as the workplace standards. Based on this work situation, it appears that the 10 ppm limit is sufficient to provide a safe working environment and the threshold for eye lacrimation is above 10 ppm limit over a large number of individuals.

In addition to this real world experience Draslovka has undertaken a complete review of all the literature on ethanedinitrile and could not find any data on eye irritation (causing long term damage to the eye as opposed to lacrimation) in any study on rats or monkeys.

Nevertheless, Draslovka agree with the change from the TWA value to ceiling value, since it ensures protection of workers from peaks of ethanedinitrile concentrations which may result in eye lacrimation. While Draslovka considers ethanedinitrile does not fall within the hazard definition described above it agrees that workers should not be exposed to the extent that lacrimation occurs.

When considering the TLV value for workers, it must be recognised that protection against lacrimation or irritation is only required for direct contact to ethandinitrile because once the

substance is removed irritation / lacrimation stops (NRC 2001 in NRC 2015). This means that the proposed value must account for the fact that the effect is not related to duration of exposure, but rather to the presence or absence of ethanedinitrile and its concentration.

The ACGIH reports have considered all available studies including the chronic toxicity and other types of toxicological data which were included in its assessment. Therefore, we propose to use TLV (WES) of 10 ppm adopted by the ACGIH between 2001 and 2015 but suggest a change to the ceiling value from the TWA to better ensure the comfort of workers.

C) TEL value

We suggest using the earlier proposed TWA for consideration of TEL value.

Although, we believe that 10 ppm would be as protective against eye lacrimation to the general population just as it is for workers, we recommend calculating the TEL by using

- the intra species uncertainty factor of 3 as proposed in the NRC (2015), and,
- the interspecies uncertainty factor of 1 since the study was done in humans.

This would derive to TEL 3.3 ppm based on TLV value.

A TEL value of 2.7 would be applied using the NRC (2014) approach i.e. calculation from 8 ppm value with no effect. The McNerney (1960) study divided the value by 3.

It is not appropriate to transform this value for different exposure durations since eye irritation due to direct contact of the substance with the eye is not likely to increase with the duration of the exposure (NRC 2001 in NRC 2014). However, both the ACGIH and the NCR (2014) did not research or question the study of McNerney (1960) itself, to understand that the study does not show eye irritation as per the regulatory hazard definition but is instead simply cause eye lacrimation.

Although other types of data are included in the ACGIH (2016) assessment it seems appropriate to consult the chronic toxicology studies to provide the TEL estimation for the general public.

This discussion must mainly focus on CN toxicity. As discussed above the NOAEC in chronic studies was estimated to be 25 ppm ethanedinitrile (≥4.7 mg and 5.2 mg CN /kg bw in monkeys and rats, respectively) and 10 mg CN /kg bw in rats as hydrogen cyanide and acetonitrile.

NCR recommends using an uncertainty factor of 2 for recalculating rat data to human rates due to the much higher sensitivity of rats toward CN (NCR 2001). Additionally, NCR (2001) recommends an uncertainty factor of 3 for recalculation of data from monkeys to humans given the high similarity of these species and the known higher sensitivity of monkeys compared to humans.

The uncertainty factor of 3 is recommended for intraspecies variation due to CN having the same toxicology and metabolism (by detoxification) that is independent of age (NCR 2001).

To set the TEL figure the chronic study on ethanedinitrile is used even though the other two studies resulted in a higher CN NOAEL. Neither of these studies reached the LOAEL. But it is noted these studies reinforce each other's findings and an additional uncertainty factor need not be applied.

TEL_{8h} = NOAEC / (UF intra* UF inter) = 25 ppm / 2 * 3 = 4.1 ppm

Since the chronic study was based on a daily exposure of 8-hours this value can be considered as resembling an 8-hour TEL or NOAEC human concentration. However, this value is higher than the 2.7 ppm calculated above which is based on the lacrimation effect and so Draslovka considers it should set the 8 hour TEL at the 2.7 ppm as the more conservative endpoint.

To calculate the 24 h TEL will be necessary to account for the fact that in the studies quoted the experimental animals were exposed to the substances for 5 days a week. This means they were exposed for only 40 hours in a 168 hour week.

Thus the 4.1 TEL set above needs to be multiplied by 40/168 to recalculate to one-week TEL (basically time weighted average). This result to 0.98 ppm, which is approximately 1 ppm.

Since this value is based on a chronic study and CN does not bio accumulate, it is reasonable to believe that this concentration may be metabolized indefinitely. Therefore, this represents the acceptable daily concentration. Due to knowledge on the sensitivity of experimental species relative to humans for CN compound inhalation toxicology and the assessment factors provided in NCR report (2001), it was not necessary to calculate the TEL value via the acceptable daily intake value.

Table 7 TEL values

	8h	1 day	1 week	annual
TEL	2.7 ppm	1 ppm	1 ppm	1 ppm

D) AEGL values discussion

Due to very steep dose response curve and very similar sensitivity of different subpopulation towards CN toxicity the question may be asked are the AEGL TEL and WES (TLV) values similar to those calculated above.

The NCR (2014) report references all available study results without questioning the quality of these studies to any extent. Consequently their approach for setting the AEGL 1 was based on a very low

quality chronic study which reported symptoms at low exposure which would normally be associated to much higher cyanide concentration (El Ghawabi 1974, ECETOC 2007).

This AEGL-1 information was than copied to the ethanedinitrile report instead of providing a value based on observed irritation. Consequently the AEGL – 2 and -3 values provide values which are inconsistent with the above chronic toxicity studies.

REFERENCES

ACGIH 2001, Cyanogen.
ACGIH 2014, Cyanogen.
ACGIH 2016, Cyanogen.
ECETOC, 2007, JACC no 53, Cyanides of Hydrogen, Sodium and Potassium, and Acetone Cyanohydrin.
NCR, 2001, Acute exposure guideline levels for selected airborne chemicals, volume 2.
NCR, 2014, Acute exposure guideline levels for selected airborne chemicals, volume 17.