All experiments reported in Table 3.3 were conducted with NaCN, but doses have been converted to equivalent mg CN^-/kg. The lethality results from experiments conducted with NaCN should be applied to other CN compounds, such as the metallo-cyanide complexes, with caution (see also Table 3.4). This is because, in addition to the absolute dose being a factor for toxicity, the rate of HCN generated in the stomach also influences the toxicity of different cyanide compounds. HCN formation is much faster from NaCN than with either WAD or other metallo-cyanide complexes (Eisler and Wiemeyer 2004, Surleva et al 2014). Fast formation of HCN from NaCN results in quick absorption of cyanide into the body. The detoxification mechanisms are not able to regenerate quickly enough to keep up with the amount of HCN absorbed (see Section 4.1) and consequently toxicity (death) results. This is not necessarily the case for metallo-cyanide complexes.

Wiemeyer et al (1986) studied the acute oral toxicity of NaCN in 6 avian species (black vulture, American kestrel, Japanese quail, domestic chicken, eastern screech-owl, and European starling). Groups of 3 to 20 male and female birds received 4 to 5 doses of sodium cyanide in gelatine capsules into the proventriculus[9]. The LD_{50} for each species was reported (Table 3.3), but only for the black vulture was sufficient information provided to indicate the lethality dose response (Table 3.1). Sublethal symptoms were similar in all species, and included slight coordination disturbance, rapid eye blinking, head-bowing, panting, lethargy and wing-droop followed by loss of coordination, convulsions and tail fanning, breathing disorders ultimately followed by death. Death occurred between 15-30 minutes after dose administration. Birds surviving past 1 hour usually totally recovered.

Table 3.1: Mortality of black vultures given a single oral dose of NaCN
(Wiemeyer et al 1986)

Dose (mg CN^-/kg)	n		Total % deaths	Time to death (min) [a]
	M	F		
1.6	2	1	0	-
2.4	2	2	25	30
3.7		3	100	15.7 (14-18)
19.1		3	100	11.3 (8-14)

M= male; F= female
[a] Mean time to death (range in parentheses).

In the Wiemeyer et al (1986) study species that are predominantly carnivores were more sensitive than those that primarily eat plant material. Although other studies in Table 3.3 clearly show ducks have lower acute LD_{50} for CN^- and are therefore more sensitive than the carnivores. Avian carnivores usually have lower digestive pH than other birds, this is to assist to break down flesh, fur or bone

[9] The first part of the stomach (i.e. the glandular stomach) after the crop.

(Donato et al 2007). The lower digestive pH may liberate more cyanide faster, and may be one of the reasons for higher susceptibility in these birds.

ECETOC (2007) describe an unpublished 5-day repeat exposure study with juvenile mallard ducks that were given nominal concentrations of 0, 100, 178, 316, 562 or 1,000 mg NaCN/L in drinking water *ad libitum* (Stence et al 1993a). Measured concentrations were considerably lower than nominal concentrations (11-26.5% of nominal) both at day 1 and day 4, and averaged 0, 21.2, 28.2, 39.6, 53.1 or 96.7 mg CN^-/L in drinking water. ECETOC (2007) do not indicate whether new cyanide solutions were made up each day or whether the same solution was used over the 5 day exposure period. No mortality was observed in the control or the two lowest dose groups, while 4 of 10 animals died at the nominal concentration of 316 mg/L (days 2 and 4) and all animals died at the two top doses (days 2 and 3). Signs of toxicity in the nominal 316 mg/L group were first noted on day 2 and included loss of coordination, lower limb weakness, and lethargy. In surviving animals these symptoms disappeared by the morning of day 6. Animals in the top two dose groups experienced reduced reaction to external stimuli, lethargy, wing drop, loss of righting reflex, prostate posture and gasping; these signs were first noted in the afternoon of day 1. No clinical signs were observed at the two lowest dose groups. However, a statistically significant dose related reduction in body weight gain was observed in all dose groups. The reduction was accompanied by a marked dose-related reduction in feed and water consumption throughout dosing and recovery periods. According to the authors, the birds receiving sodium cyanide reduced their water consumption to a point where many of the deaths may well have been due to dehydration rather than treatment alone. Based on the original nominal concentrations, the 5-day LD_{50} was reportedly determined to be 340 mg NaCN/L (180 mg CN^-/L) or approximately 75 mg/kg bw using the average drinking water consumption (415 ml/kg bw) of the nominal 316 mg NaCN/L group. Because the measured concentrations were considerably lower, ECETOC (2007) considered it more appropriate to base the evaluation on the measured concentrations. According to ECETOC (2007) the LD_{50} was approximately 18 mg CN/kg bw, this corresponded to an approximate water concentration of 43 mg CN^-/L and the low observed effect level (LOEL) was approximately 21.2 mgCN^-/L (i.e. 19 mg CN^-/kg bw). The calculated doses for the LD_{50} and LOEL are at odds with each other, since the LD_{50} should be higher than the LOEL. It is of note that mortality in this experiment was not observed shortly after drinking one dose of NaCN in water as it was with most acute experiments, but rather it occurred a few days after the first administration. The measured concentrations of CN^- in water in this study were much lower (approximately a factor of 10 or more) than the nominal concentrations. This creates uncertainties in the concentrations of CN^- to which the birds were actually exposed, therefore limiting the usefulness of this study.

The same authors conducted a similar experiment with the same study design but in juvenile northern bobwhite quails (*Colinus virginianus*) (10 days of age) (Stence et al 1993a, described in ECETOC

2007). Quails (10/group) received nominal concentrations of 0, 100, 178, 316, 562 and 1,000 mg NaCN/L (0, 53, 94, 168, 298, 530 mg CN^-/L)[10] in their drinking water for 5 consecutive days, followed by 3 days observation. The results of the study are summarised in Table 3.2.

Table 3.2: Mortality and clinical signs in juvenile northern bobwhite quail exposed *ad libitum* to NaCN in drinking water for 5 days

Nominal CN concentration in water (mg CN^-/L) [a]	Converted dose (mg CN/kg)	% deaths [c]	Signs of toxicity
0	0	0	None
53	33 [b]	0	None
94	-	0	Wing drop, lethargy, ↓ body weight gain, ↓ food & water consumption
168	35 [b]	0	As above plus depression & reduced reaction to external stimuli
298	-	10	As above plus loss of coordination
530	-	100	Presumably as above

Data from Stence et al (1993a) as summarised in ECETOC (2007)
.- = none reported.
[a] Analytical measurement of the test solutions confirmed the nominal concentrations with the exception of the 94 mg CN^-/L at day 5, where the sample only contained 9.1% of the nominal concentration.
[b] ECETOC (2007) appears to have determined the intake doses based on body weight and average drinking water consumption rates over the test period. Because drinking water consumption was reduced by concentrations ≥94 mg CN/L, the converted mg/kg doses between the highest concentration at which no clinical signs were observed (53 mg CN^-/L) and the highest concentration at which no mortality was observed (168 mg/L) are almost the same.
[c] Number of treated birds was 10.

Clark et al (1991)[11] determined the acute (24-hour) oral toxicity of NaCN to a number of species. It is not stated whether exposure was via gavage or drinking water. Mallard ducks were most sensitive (LD_{50} 1.5 mg CN^-/kg) followed by little brown bats (4.5 mg CN^-/kg), house mice (4.6 mg CN^-/kg), and white-footed mice (14.9 mg CN^-/kg) (see Table 3.3). According to the authors, slopes of the dose response curves were extremely steep. Unlike other species little brown bats showed delayed mortality (Clark et al 1991).

In another study, groups of wild possums were dosed with NaCN by oral gavage, the LD_{50} determined to be 4.6 mg CN^-/kg (Bell 1972).

[10] Analytical measurement of test solutions confirmed the nominal concentrations with the exception of the 178 mg NaCN/L sample at day 5, which had only 9.1% of the nominal concentration.
[11] The paper is an abstract presented at a conference and only limited information is provided.

Sterner (1979) found oral application of a number of NaCN doses to the back of the mouth of coyotes (1 coyote per dose) resulted in death of the animals within 5-41 minutes at doses ≥ 2.2 mg CN^-/kg. The LD_{50} for all the organisms discussed above are summarised in Table 3.3. ECETOC (2007) and NICNAS (2010) concluded concentration levels protective of birds would also be protective of other animals (e.g. bats and terrestrial vertebrates).

Table 3.3: Oral LD_{50}s and $NOEL_{mortality}$ for sodium cyanide (as CN^-) in wildlife

Species	Dosing regime	LD_{50} (mg CN^-/kg bw) [a]	$NOEL_{mortality}$ (mg CN^-/kg bw) [a]	Source
Birds				
Mallard duck (*Anas platyrhynchos*)	Bolus dose gelatine capsule	1.4	0.53	LD_{50} from Henny et al 1994. $NOEL_{mortality}$ from Hagelstein and Mudder 1997a, as cited in NICNAS 2010.
	Not specified	1.5	-	Clark et al 1991, as cited in ECETOC 2007.
	Single dose in tap water[b]. *(181 mg total CN/L)*	1.3 [e]	-	Fletcher 1986, as cited in NICNAS 2010.
	Single dose of effluent *(212 mg total CN/L)* pond water	1.7 [e]	-	
	Ducks kept on water at different CN conc's for 5 days.	18 [d] *(43 mg CN/L)*	16.4 [d] *(28 mg CN/L)*	Stence et al 1993a, as cited in ECETOC 2007.
American kestrel (*Falco sparverius*)	Bolus dose gelatine capsule	2.1	-	Wiemeyer et al 1986
Black vulture (*Coragyps atratus*)	As above	2.5	1.6	
Eastern screech owl (*Otus asio*)	As above	4.6	-	
Japanese quail (*Coturnix japonica*)	As above	5.0	-	
European starling (*Sturnus vulgaris*)	As above	9.0	-	
Domestic chicken (*Gallus domesticus*)	As above	11.1	3.2	
Northern bobwhite quail (*Colinus virginianus*)	Dosing over 5 days in drinking water	69.0 *(374 mg CN/L)*	35 [f] *(168 mg CN/L)* 33 [f] *(53 mg CN/L)*	Stence et al 1993b, as cited in ECETOC 2007

Species	Dosing regime	LD_{50} (mg CN^-/kg bw) [a]	$NOEL_{mortality}$ (mg CN^-/kg bw) [a]	Source
Mammals				
Brown bats (*Myotis lucifugus*)	Not specified	4.5	-	Clark et al 1991
House mice (*Mus musculus*)	Not specified	4.6	-	
White-footed mice (*Peromyscus leucopus*)	Not specified	14.9	-	
Laboratory rat (*Rattus norvegicus*)	Not specified	2.7 – 8 (Table 4.1)	-	Various (Table 4.1)
Common brushtail possum (*Trichosurus vulpecula*)	Oral gavage	4.6	-	Bell 1972
Coyote (*Canis latrans*)	To back of mouth	2.2 [c]	-	Sterner 1979

- $NOEL_{mortality}$ not reported and could not be ascertained from the given information.
LD_{50} = median lethal dose; $NOEL_{mortality}$ = highest dose at which no mortality was observed.

[a] All experiments listed in this table were conducted with NaCN. For this table, doses reported in the publications have been converted to equivalent doses of cyanide ion, i.e. mg CN^-/kg body weight. Concentrations provided in the paper were converted by ToxConsult to equivalent. The experimental NaCN was divided by a conversion factor of 1.88.

[b] NICNAS (2010) indicate it is not clear whether this was administered by gavage, or was in fact simply provided on a single occasion for a limited period in drinking water.

[c] Only one coyote per dose group. Coyotes given doses ≥ 2.2 mg CN^-/kg died.

[d] The doses in the table for the Stence et al. (1993a) study were calculated by ECETOC (2007) using measured water concentrations. The measured concentrations of CN^- in water in this study were lower (by approximately a factor of 10 or more) than the nominal concentrations and as a result provide a more conservative estimation of the effect concentrations of cyanide than if the nominal concentrations were used in the calculations. Because the assumptions for calculating the doses from the measured water concentrations were not provided by ECETOC (2007), there is some uncertainty in the resulting doses.

[e] The original study report (Fletcher 1986) is unpublished and was not available to NICNAS (2010) or ToxConsult when summarising the information. NICNAS (2010) drew on the discussion of the study in secondary sources which presented few details of the test methodologies and data. It is not clear, for example, whether the dose was administered by gavage or simply provided on a single occasion for a limited period in drinking water. Sublethal effects were not reported in the data available to NICNAS (2010).

[f] The deaths were observed at a nominal water concentration of 168 mg CN^-/L, therefore the dose corresponding to this water concentration (35 mg CN^-/kg/d) represents a $NOEL_{mortality}$ in this study. No effects (sublethal or lethal) were observed at a water concentration of 53 mg CN/L, therefore the dose corresponding to this concentration represents a true $NOEL_{any\ effect}$ (i.e. 33 mg CN^-/kg/d). ECETOC (2007) appears to have determined the intake doses based on body weight and average drinking water consumption rates over the test period, but does not provide the details of their assumptions. Because drinking water consumption was reduced by concentrations ≥94 mg CN^-/L, the converted mg/kg doses between the highest concentration at which no clinical signs were observed (53 mg CN^-/L) and the highest concentration at which no mortality was observed (168 mg/L) are almost the same. Birds given 94 mg CN/L exhibited wing drop and lethargy, and those given 168 mg CN/L exhibited depression and reduced reaction to external stimuli. At 298 mg CN/L one mortality (1/10) was recorded and loss of coordination was observed in all birds in addition to symptoms observed at lower doses. All (10/10) birds died at the top dose (530 mg CN/L) (see also Table 3.2).

In a field study, Henny et al (1994) observed bird deaths at 2 out of 16 tailings ponds at gold mines in Nevada.

- The WAD at the discharge point in these 2 tailings ponds was 81 and 62 mg/L (pH 10.3 and 10.6).
- The three mines with the highest WAD concentrations at the discharge point (138-216 mg/L) had no birds present (live or dead) during the investigators' visits. WAD concentrations at the other tailings ponds ranged from 8.4 to 59 mg/L.
- Detailed field observations were made at one tailings pond, at which bird deaths had previously been reported by mining personnel (Henny et al 1994). The reclaim end of the pond, where a recent dead duck was found, had total CN concentrations of 196-207 mg/L (WAD not reported). The investigators recorded their observations for three green-winged teal (*Anas crecca*), who landed and almost immediately began drinking water from the same pond. All birds exhibited excessive lateral bill shaking within 3 minutes but appeared normal within 15 minutes as they swam into a small flowing channel on the delta formed by the discharge. An hour later and for about 5 hours, the teal appeared somewhat lethargic in the water, moving slowly and/or sleeping (they also did not drink water during this time) until they appeared to fully recover before leaving the pond area.
- At another tailings pond, 13 ducks of several species swam near the reclaim area (WAD 19 mg/L) for 2 hours without evidence of intoxication, and appeared normal when they flew away.
- Five cinnamon teal (*Anas cyanoptera*) landed on a pond in the delta area near the tailings discharge (WAD 62 mg/L). One of the four males drank liberally and soon began splashing its head in the water and shaking its bill laterally. Within about 9 minutes of arrival, an additional male and female also appeared to drink smaller amounts, and within 13 minutes all birds had drunk. However the first male clearly appeared to have drunk the most. About 16 minutes after arrival, the first male lost control of its neck and lowered its head into the water. After about 10 minutes of at least partial submersion, it began flapping its wings in the water, moving forward but could not raise its bill out of the water. About 32 minutes after landing, the male was recorded as dead. The surviving birds behaved normally until flushed by a shell-cracker after about 100 minutes on the pond. They then flew to a freshwater pond about 1 km away. Two hours later what appeared to be the same four teal were observed at the freshwater pond and were normal.

Henny et al (1994) also carried out an experiment where adult mallards were kept for 4 hours in tanks containing 115 mg CN^-/L as NaCN adjusted to pH 10.5. Some birds exhibited lateral bill shaking soon after drinking the water and repeated the response after other drinking bouts. After initial bill shaking, some birds were alert, others stupefied, and others arched their neck with the bill pointing upward and

appeared to gasp. The latter posture was often associated with wing extension and a burst of powerful flapping which would abruptly cease with death or a stupefied appearance. During stupefaction, the head often drooped into the water and sometimes remained submerged without struggle until death. Stupefied birds which did not die usually roused in 15-30 minutes, appeared alert, began drinking and generally repeated the same behavioural sequence, with the probability of death from the second exposure highly dependent on how much water was drunk. Although many experimental birds experienced multiple periods of stupefaction, if they survived the initial two exposures, death rarely occurred after three cycles or after about 1.5 hours of the 4 hour trial (Henny et al 1994). It is apparent the birds did not develop an aversion to drinking the cyanide water despite multiple times of intoxication.

Henny et al (1994) also conducted laboratory studies where mallard ducks were given a bolus dose of NaCN by gelatine capsule. This gave an oral LD_{50} of 1.4 mg CN^-/kg bw and a $NOEL_{mortality}$ of 0.53 mg CN^-/kg bw (Table 3.3).

Link et al (1996) also conducted acute toxicity studies with mallard ducks using different forms of cyanide, these are further described in Section 3.1.1.2.

Non-lethal effects:

- Two authors have investigated acute sublethal biochemical effects of KCN on adult female mallards (Pritsos and Ma 1997, Ma and Pritsos 1997). KCN solutions were administered at a consistent volume (10 mL) via gavage at doses of 0, 0.1, 0.2, 0.4 or 0.8 mg CN^-/kg bw. Significant depletions of heart, liver and brain tissue ATP were observed at all doses at the first monitoring point (2 hours after exposure); these levels returned to normal by 24 hours post-exposure. Significant decreases in respiratory control ratios in liver, brain and heart were observed from 0.2 mg CN/kg bw, and a significant increase in serum creatine, rhodanese and 3-mercaptopyruvate sulphurtransferase enzyme activities were noted in the brain at 0.4 mg CN/kg bw.

- Cooper (2003, as cited in NICNAS 2010) investigated pigeon flight times after orally dosing them with a KCN solution at 0, 0.4, 0.5 or 0.8 mg CN/kg bw (10 mL at a concentration of 50-80 mg CN/L on a single occasion) and allowing them to recuperate for 15-20 minutes prior to flying. A dose dependent response was found, with significantly longer flights at the two highest doses.

- Female quails (n=27) were gavage dosed with 0, 1 or 3 mg KCN/kg bw/day (i.e. 0, 0.4, or 1.2 mg CN/kg/d) for 7 consecutive days (Rocha-e-Silva et al. 2010); one quail dosed with 3 mg KCN/kg bw/day developed moderate trembling and vocalisation after the first day of dosing, which progressed to convulsions and death on the second day. No effects were observed in other birds, including on body weight, body weight gain, food consumption, or macroscopic lesions.

3.1.1.2 Other forms of cyanide

Link et al (1996) replicated the acute bolus oral toxicity study in mallard ducks originally conducted with NaCN by Henny et al (1994) but with different cyanide compounds (KCN, CuCN, $Hg(CN)_2$ and $CH_2(CN)_2$), but used 14 birds per toxicant instead of the 72 used by Henny et al (1994). The birds in the Link et al (1996) study were administered an amount (via bolus dose) equal to the cyanide content of the sodium cyanide LD_{50} from the Henny et al (1994) study (1.4 mg CN^-/kg). Nine of the 14 birds bolus dosed with KCN died, whereas no birds died with the other toxicants (Table 3.4), indicating the KCN salt was as similarly toxic to NaCN but the other compounds were significantly less toxic. Interestingly, when Link et al (1996) exposed birds to contaminated water over a 2-hour period, deaths occurred in the $Hg(CN)_2$ group but not in the other groups. The study authors did not provide information on whether both groups drank the water during the exposure period. The results of this study suggest there is uncertainty in extrapolating the results from experiments conducted with NaCN or KCN to other CN compounds, such as metallo-cyanide complexes. Particularly interesting is that zero deaths were recorded with copper cyanide both after acute bolus dosing and exposure to contaminated water for 2 hours.

Table 3.4: Acute toxicity in mallard ducks for different cyanide compounds
(Link et al 1996)

Cyanide compound	Number of bird deaths (out of n=14) [a]	
	Oral	Water
KCN	9	6
CuCN	0	0 [b]
$Hg(CN)_2$	0	13
$CH_2(CN)_2$	0	0

[a] Birds were administered the test compound in an encapsulated form (bolus oral dose) or placed in pens with contaminated water and exposed for a 2-hour period.
[b] The compound is not water soluble so a water soluble substitute, $Na_2Cu(CN)_3$, was used for this exposure route.

It has been speculated the rapid recovery of some cyanide-exposed birds in acute experimental studies may be due to the quick detoxification of absorbed HCN to thiocyanate (Eisler and Wiemeyer 2004). It is possible some birds drinking at cyanide tailings ponds may not die immediately after

drinking lethal cyanide solutions, and instead could die later if they fly away soon after drinking the water (ECETOC 2007, Donato et al 2008, NICNAS 2010).

NaCN rapidly forms free cyanide in the avian digestive tract (pH 1.3-6.5), but the formation of free cyanide from WAD or other metal cyanide complexes is comparatively slow (Eisler and Wiemeyer 2004). A high rate of cyanide absorption is critical to rapid acute toxicity[12], and absorption may be delayed by the lower dissociation rates of metal-cyanide complexes (Hagelstein and Mudder 1997b, in NICNAS 2010; Eisler and Wiemeyer 2004). Because HCN from the WAD fraction may become available sometime after being ingested, regulations typically require measurement of total cyanide and/or WAD cyanide in mine effluents rather than, or in addition to free cyanide.

3.1.1.3 International Cyanide Management Code

The International Cyanide Management Institute (ICMI 2012) developed the International Cyanide Management Code (ICMC), a voluntary initiative for the gold mining industry and for producers and transporters of cyanide used in gold mining. The Code is intended to complement an operation's existing regulatory requirements, and focuses exclusively on the safe management of cyanide produced, transported and used for the recovery of gold, included is cyanide that may be in mill tailings and leach solutions.

The Code itself contains brief overarching principles and standards of practice. Standard of Practice number 4 requires signatories to:

> "*Manage cyanide process solutions and waste streams to protect human health and the environment.*"

It states a signatory should:

> "*Implement measures to protect birds, other wildlife and livestock from adverse effects of cyanide process solutions.*"

The ICMI have published a companion document to the Code which provides implementation guidance for the Standards of Practice contained in the Code (ICMI 2009). The guidance states a concentration of 50 mg/L WAD cyanide, or lower, in solution is typically viewed as protective against mortality of most wildlife and livestock. However the basis of this statement is not provided in either

[12] This is because the inherent detoxification processes will only be overwhelmed if the dose is high enough and HCN formed in the GIT is absorbed quickly. If the bird quickly leaves the tailings facility not enough HCN may have been absorbed to cause disablement. Similarly relatively slow release of HCN from WAD may mean a disabling amount of HCN is absorbed over time, initially detoxification mechanisms may be able to cope with the influx of HCN but may not recover quickly enough if the WAD dose was large.

ICMI documents. The guidance also indicates where birds, wildlife or livestock have access to cyanide containing water measures should be taken to limit WAD cyanide concentration to a maximum of 50 mg/L.

An appraisal of the scientific literature undertaken for this toxicity profile suggests the value of 50 mg/L WAD may have basis in observations and incident reports at cyanide containing tailings ponds where avian mortalities had been observed. From the limited information available, it seems a WAD cyanide concentration > 50 mg/L was associated with bird deaths, but at sites where WAD was generally < 50 mg/L, few or no mortalities were observed (MERG 2001, NICNAS 2010, DRET 2008, NT DoME 1998, NPS 1997, Donato et al 2008, Griffiths et al 2014a, Hudson and Bouwman 2009). Some of this information has come from studies investigating bird visitations to tailings storage facilities in the Northern Territory, West Australia and Queensland (NT DoME 1998, Donato et al 1997, 2007, 2008, Donato and Smith 2007, Donato 1999). Since these were industry commissioned studies, reports containing the raw data were not located. Henny et al (1994) reported bird and wildlife deaths at tailings ponds and heap leach facilities in Nevada containing concentrations of WAD of 62 mg/L, 81 mg/L and higher, but not at those containing <50 mg/L WAD CN.

3.1.2 Inhalation toxicity

Apart from oral exposure (Section 3.1.1), wildlife may also be potentially exposed by inhalation of HCN at the surface of the tailings dam. Compared to the considerable amount of information available for the oral route, there is little on inhalational toxicity of HCN in avian species.

Barcroft (1931) exposed chickens, pigeons and canaries to various air concentrations of HCN and determined the relationships between concentration and time of exposure. When all three bird species were exposed to 120 mg HCN/m^3, chickens survived for at least 60 minutes (the maximum time recorded), while pigeons and canaries died within 10 and 3 minutes, respectively. The total number of birds that died is not clear from the graphical data in the paper.

Barcroft (1931) also exposed three groups of 4 goats to a nominal concentration of 360 mg HCN/m^3 for 15, 20 or 24 minutes. The number of goats that died were 1 (at 15 minutes), 3 (at 20 minutes) or 4 (at 24 minutes).

Several studies are available which have determined LC_{50} and LC_{01} values for HCN for a number of exposure periods in rats, rabbits, and mice. The studies are summarised in Table 3.5. It is evident from the table that the inhalation LC_{50} decreases as exposure time increases. In the Higgins et al (1972) study conducted with mice and rats, all deaths occurred during or 20 minutes after the

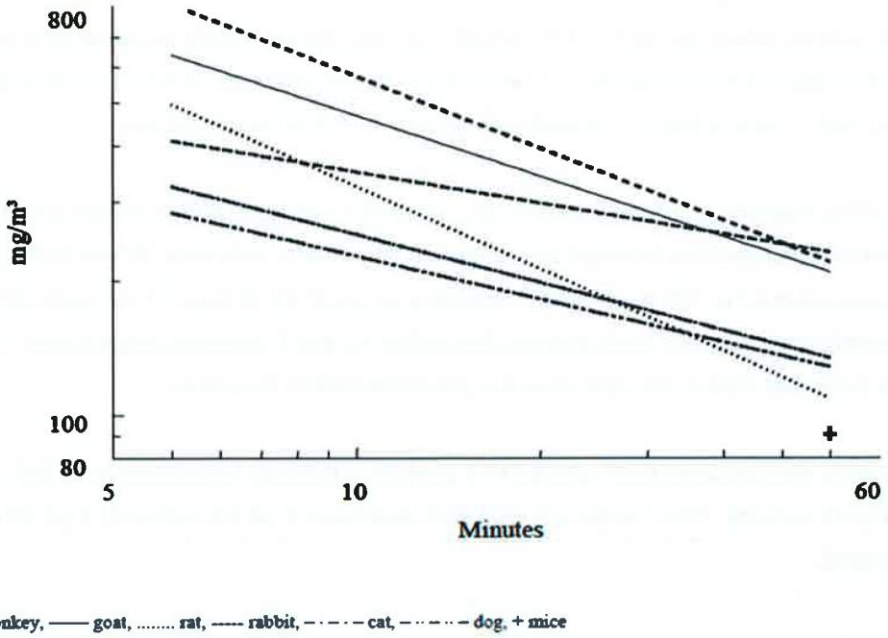
exposure period, there were no delayed deaths. This may be different for bats, since after oral dosing their death was delayed (Clark et al 1991). Studies investigating nonlethal inhalation toxicity in monkeys, rats and mice are summarised in Table 3.6. The primary effects are those associated with decreased function of tissues with high metabolic demand (i.e. the central nervous system and the heart). Respiratory effects, secondary to CNS depression, are commonly observed before incapacitation.

ECETOC (2007) reviewed the concentration - time relationship for HCN lethality in detail for a number of species (rat, rabbit, cat, dog, goat, monkey). ECETOC (2007) concluded concentrations required for lethality were lower for longer inhalation exposure times. Figure 3.1 summarises the concentration-time relationships for concentrations causing 50% mortality. The regression equation for rats is:

$$P = b \times \ln (C^N \times t) - a]$$

Where:

P = Probit.
C = HCN concentration (mg/m^3).
t = Exposure time minutes.
N = 1.64
b = 0.701
a = 3.27



* - - - - Monkey, —— goat, ... rat, ----- rabbit, – · – · – cat, – · · – · · – dog, + mice

Figure 3.1: Concentration – time relationships for various species for 50% mortality
Figure is reproduced from ECETOC (2007).

Table 3.5: Inhalation lethality data for HCN exposure in terrestrial mammals

Mammal	Exposure time (min)	LC_{50} (mg HCN/m^3) [a]	LC_{01} (mg HCN/m^3) [a]	$NOEC_{mortality}$ (mg HCN/m^3) [a]	Source
Rat	0.2	3,800	-	-	Ballantyne 1983 [d]
	1	1,480	-	-	
	5	556	-	313	Higgins et al 1972
	5	535	-	-	Vernot et al 1977
	5	496	-	-	Ballantyne 1983 [d]
	5	408	313	302	Du Pont 1981 [d]
	15	217	153	122	
	30	221	-	-	Kimmerle 1974 [d]
	30	191	140	-	Du Pont 1981 [d]
	30	177 [b]	-	-	Levin et al 1987
	30	174	-	-	Ballantyne 1983 [d]
	60	159	-	-	
	60	154	97	84	Du Pont 1981 [d]
	60	133	-	-	Kimmerle 1974 [d]
Mouse	5	357	-	221	Higgins et al 1972
	30	183 [c]	-	-	Matijak-Schaper and Alarie 1982 [d]
Rabbit	0.01	2,446	-	-	Ballantyne 1983 [d]
	5	415	-	-	
	35	209	-	-	

- = $NOEC_{mortality}$ not reported and could not be determined from the given information.

[a] If concentrations were reported in ppm HCN in the paper, these have been converted by ToxConsult to equivalent mg/m^3 concentrations of HCN using the following standard formula: (ppm x molecular weight HCN) ÷ 24.45

[b] Head-only exposure.

[c] Mortality ratios for the mice (n=4 per group) were 0/4, 2/4, 3/4 and 4/4 for exposure to concentrations of HCN at 111, 166, 243 and 365 mg/m^3 respectively. The recovery period was 10 minutes, during which the surviving mice appreciably recovered.

[d] As cited in NRC 2002.

Table 3.6: Non-lethal toxicity data for HCN exposure in terrestrial mammals

Mammal	Exposure time (min)	Concentration (mg HCN/m^3) [b]	Effect	Source
Monkey	12	138	"Distinctly toxic"	Dudley et al 1942 [f]
	19	111	Time to incapacitation [c]	Purser et al 1984
	16	113		
	15	136		
	8	162		
	8	172		
	30	66	Slight ↑ respiratory minute volume, EEG evidence for CNS depression	Purser 1984
Rat	5	302	No toxic signs	Du Pont 1981 [a, f]
	12.5	221 [d]	↑ cardiac-specific creatine phosphokinase activity & ↑ ectopic heart beat after norepinephrine injection	O'Flaherty and Thomas 1982

Mammal	Exposure time (min)	Concentration (mg HCN/m^3) [b]	Effect	Source
	30	61	↑ transthoracic pressure, ↑ air flow and tidal volume, ↓ compliance, respiratory rate & minute volume, ↓ lung phospholipid level	Bhattacharya et al 1994
	960	18	No deaths, no overt toxic signs	Weedon et al 1940 [f]
Mouse	30	70	50% respiratory depression [e]	Matijak-Schaper and Alarie 1982 [f]
	30	111	No mortality or overt toxic signs (e.g. unconsciousness)	
	960	18	No deaths, no overt toxic signs	Weedon et al 1940 [f]
	1,440	33	Pulmonary congestion	Pryor et al 1975 [f]
	5	137	Incapacitation	Sakurai 1989 [f]
	10	82		
	20	24		
	30	46		

[a] Animals in the Du Pont (1981) studies were observed for 14 days after exposure.
[b] If concentrations were reported in ppm HCN in the paper, these were converted by ToxConsult to equivalent mg/m^3 concentrations of HCN using the following formula: (ppm x molecular weight HCN) ÷ 24.45
[c] Semi-conscious state with loss of muscle tone.
[d] Nose-only exposure.
[e] According to NRC (2002), the description given by Matjak-Schaper and Alarie (1982) indicates the concentration of 70 mg HCN/m^3 appears to be the threshold for a breathing pattern characteristic of asphyxiation.
[f] As cited in NRC 2002.

3.1.3 Dermal toxicity

Wildlife exposure to cyanide in tailings may also potentially occur dermally, especially for water birds. The lethal dermal dose depends upon the area of skin exposed, the condition of the skin and length of time a substance is on the skin (ECETOC 2007).

It is apparent HCN readily penetrates intact mammalian skin but cyanide salts are less able to enter the systemic circulation after being placed on the skin. As expected the compounds are more toxic with abraded skin (Table 3.7). Times to the onset of toxic signs varied between 5 minutes and 1 hour with HCN. With abraded skin, the times to intoxication were shorter. With NaCN and KCN, time to onset of toxicity ranged from 15 minutes to 4 hours. Times to death were also variable, and ranged from 15 minutes to 6 hours. The study did not report the surface area of skin to which the cyanide was applied (Ballantyne 1987, as cited in ECETOC 2007).

Table 3.7: Dermal LD_{50}s cyanide exposure in rabbits [a]

Cyanide compound	Skin condition	LD_{50} (mg CN/kg bw) [b]
HCN (solution)	Intact	6.9
	Abraded	2.3
KCN (solution)	Intact	22.3
	Abraded	14.3
NaCN (solution)	Intact	14.6
	Abraded	11.3
NaCN (moist)	Intact	11.8
NaCN (powder)	Intact	>200
	Abraded	7.7

[a] Source: Ballantyne 1987, as cited in ECETOC 2007
[b] $NOEL_{any\ effect}$ and $NOEL_{mortality}$ not reported or could not be ascertained from the given information.

Studies investigating the dermal toxicity of cyanide to birds or bats were not found, the relevance of data in Table 3.7 to birds is uncertain. The bare skin on bird legs has a markedly greater horny layer compared to dorsal rabbit skin.

3.2 Aquatic organisms

Aquatic organisms are very sensitive to cyanide. Fish are the most sensitive, followed by invertebrates (MERG 2001, NICNAS 2010, NPS 1997, Eisler and Wiemeyer 2004). Algae and aquatic plants are comparatively tolerant to cyanide. As in mammals, in fish cyanide inhibits aerobic metabolism by irreversibly binding to the ferric ion in the haem moiety of cytochrome oxidase. As indicated previously, HCN is the principal toxic form of cyanide to aquatic organisms, as it readily crosses biological membranes.

Free cyanide is the most appropriate indicator for aquatic organisms (Redman and Santore 2012) consequently guidelines protecting aquatic organisms are consistently derived for free cyanide, rather than total cyanide or WAD. Experiments investigating the toxicity of metal-cyanide complexes to fish have shown metal complexes to be significantly less toxic than free cyanide, and toxicity decreases with increasing stability of the metallo-cyanide complex (NICNAS 2010, ANZECC 2000, NPS 1997, Little et al 2007).

Acute toxicity of free cyanide to fish as 96-hour LC_{50}s (i.e. concentrations lethal to 50% of the test population) ranges between 27 and 1,200 µg/L (ECETOC 2007, ANZECC 2000). Saltwater species

are generally less (by a factor of 2) sensitive than freshwater fish. The dose-response curve for lethal effects is very steep. Cyanide concentrations without mortality (LC_0) or up to 10% mortality (LC_{10}) are close to the LC_{50}, especially for sensitive species such as rainbow trout (ECETOC 2007).

Acute toxicity to invertebrates shows wide variation between species, with LC_{50} or EC_{50} (i.e. concentrations having a particular biological or biochemical effect on 50% of the test population) ranging from 30 to 2,200 µg/L (ECETOC 2007, ANZECC 2000). Median effective concentrations (i.e. EC_{50}s) for algae range from 45 to >500 µg/L (ECETOC 2007).

In chronic toxicity studies, reliable No Observed Effect Concentrations (NOECs) range from 1 to 29µg/L for fish, 3.9 to 30 µg/L for invertebrates, and 3.9 to 700 µg/L for algae (ECETOC 2007, Eisler and Wiemeyer 2004). Sublethal effects of cyanide on fish include impaired swimming and reproduction (e.g. spawning, egg production, spermatogenesis) (NICNAS 2010, NPS 1997, Eisler and Wiemeyer 2004).

A range of acute and chronic toxicity data are available for un-ionised HCN and were considered in the development of the ANZECC water quality guidelines (ANZECC 2000). The cyanide freshwater trigger value for protection of 95% of aquatic species is 7 µg CN/L and 4 µg CN/L for marine species. Both trigger values are considered by ANZECC (2000) to be of moderate reliability.

ECETOC (2007) also used a species sensitivity distribution approach for estimating a predicted no effect concentration (PNEC) for freshwater and saltwater species. Based on the 5th percentile of the distribution (i.e. 95% species protection level) populated using No Observed Effect Concentrations for sublethal effects on aquatic species, ECETOC (2007) derived a PNEC of 1 µg/L for free cyanide.

3.3 Terrestrial plants

By comparison to other organisms, terrestrial plants seem to be resistant to cyanides (ECETOC 2007, NICNAS 2010) and certain plants may themselves be sources of cyanide in the form of cyanogenic glycosides (see Section 2.4). Short-term exposure of roots to concentrations in excess of 1 mg CN^-/L in water seems to be tolerated well in most cases. HCN has been used successfully at concentrations in excess of 1,124 mg/m^3 for the fumigation of different types of nutrient grain (ECETOC 2007). Cyanide phytotoxicity decreases with cyanide-metal complexation and associated stabilisation, particularly iron-complexed cyanides (NICNAS 2010). For example, phytotoxicity tests conducted with nutrient solutions containing iron-complexed cyanide or cyanide salts found concentrations of 1,000 mg/L were non-toxic to poplars (Trapp and Christiansen 2004).

ToxConsult

3.4 Key Points from Section 3

- *Cyanides are not persistent in the environment, do not bioconcentrate and are not bioaccumulative.*

Birds and wildlife

- *There is a body of evidence from various anecdotal and scientific observations and incident reports at mine sites indicating significant avian mortalities have occurred when WAD CN concentrations are >50 mg/L, but relatively few or no mortalities at lower concentrations.*

Oral toxicity:

- *From repeat oral exposure data in birds, according to Australian authorities (NICNAS) 50 mg/L WAD equates to an estimated overall bird lethality of approx 1% and provides acceptable protection for birds that may drink the water.*
- *Experimental studies where birds were exposed to cyanide in drinking water or via oral bolus doses give avian LD_{50} values ranging from 1.4 to 69 mg CN^-/kg bw. No Observed Effect Levels (at which no mortalities occurred) ranged from 0.53 mg CN/kg bw for mallard ducks to 6 mg CN/kg bw for domestic chickens.*
- *Experimental toxicity studies in birds indicate KCN is similarly toxic in terms of lethality after oral exposure as NaCN but other compounds, particularly metallo-cyanides such as copper cyanide, are significantly less toxic.*
- *Water WAD concentrations protective of birds are also considered by Australian and European authorities to be protective of bats and terrestrial vertebrates (e.g. reptiles and macropods).*

Inhalation toxicity:

- *There is little information available for the inhalation toxicity of HCN to birds.*
- *Several studies have determined LC_{50} values for HCN for a variety of exposure periods in rats, rabbits, and mice. Lethality is dependent upon the HCN concentration and time of exposure. LC_{50} values ranged from 133 (rat, 60 mins) to 3,800 mg HCN/m^3 (rat, 12 sec).*
- *No Observed Effect Concentrations (for lethality) were only available in a few studies, and ranged from 84 (rat, 60 mins) to 313 mg HCN/m^3 (rat, 5 mins).*
- *Other studies have investigated sublethal effects of inhaling HCN in monkeys, rats and mice. Sublethal effects were characteristic of respiratory depression and worsened with increasing HCN concentrations in air.*

Dermal toxicity:

- *HCN readily penetrates intact and damaged mammalian skin. Data were not available for birds.*

Tolerable water concentrations:

- *ECETOC (2007) derived a "tolerable" water concentration for protecting wildlife of 2mg CN^-/L (free cyanide) by applying an assessment factor of 10 to the Low Observed Effect Level (for decreased water intake and decreased body weight) of 21.2 mg CN^-/L from an experimental study with mallard ducks exposed in drinking water for 5 days.*
- *NICNAS (2010) used data from acute oral toxicity tests to derive a predicted no effect concentration (PNEC) for CN^- of ~1mg/L, but indicated this low PNEC may not be justified based on field evidence and difficulties with extrapolating acute toxicity data from laboratory studies to risk in the field. The derivation of this PNEC is not described by NICNAS and therefore there is large uncertainty in its appropriateness.*

Aquatic organisms

- *Aquatic organisms are sensitive to cyanide, with fish being the most sensitive, followed by invertebrates. Algae and aquatic plants are comparatively tolerant to cyanide.*
- *In chronic toxicity studies, reliable NOECs for free cyanide range from 1 to 29µg/L for fish, 3.9 to 30 µg/L for invertebrates, and 3.9 to 700 µg/L for algae.*
- *The ANZECC freshwater trigger value for protection of 95% of aquatic species is 7 µg CN/L.*
- *ECETOC (2007) also used a species sensitivity distribution approach and selected the 5^{th} percentile of the distribution (i.e. 95% species protection level) to estimate a PNEC of 1 µg/L of free cyanide for freshwater and saltwater species.*

Terrestrial plants

- *By comparison to other organisms, terrestrial plants seem to be relatively resistant to cyanides and certain plants themselves may be sources of cyanide in the form of cyanogenic glycosides.*

4. Human toxicity

4.1 General information

Cyanide is a potent and rapid-acting asphyxiant by inhibiting the enzyme cytochrome C oxidase, it chemically blocks the utilisation of oxygen and the production of adenosine 5'-triphosphate (ATP). Tissues with high metabolic demands such as the central nervous system and heart are therefore key tissue targets (ATSDR 2006, HPA 2011, WHO 2004, NPS 1997, WHO 1997). Cyanide also has potential secondary goitrogenic effects[13] via thiocyanate (SCN^-), the detoxification metabolite of cyanide. In high enough concentrations and for long enough, this metabolite may inhibit iodine uptake by the thyroid (Gezondheidsraad 2002, WHO 2004, ECETOC 2007).

The toxicity of individual cyanide compounds is dependent on the ease with which they release free CN^- to form HCN within the gastro intestinal tract (HPA 2011, ATSDR 2006). The cyanide in WAD is less toxic than the simple salts (e.g. sodium cyanide) and HCN. The strongly complexed metallo-cyanides (e.g. iron cyanides) are practically non-toxic (ATSDR 2006).

HCN is readily absorbed by humans after inhalation, oral and dermal exposure (Gezondheidsraad 2002, ATSDR 2006, WHO 2004, US EPA 2010, DEFRA 2002, NPS 1997). The onset of effects is quickest with inhalation and ingestion, but longer for the dermal route. Once absorbed, cyanide is rapidly and ubiquitously distributed throughout the body, although the highest levels are typically found in the liver, lungs, blood and brain (Gezondheidsraad 2002, WHO 2004).

Because HCN is a weak acid, the acidic environment in the stomach favours the non-ionised form and hence HCN formation from the CN^- from dissociation of the simple salts. The non-ionised form is also favoured under neutral pH conditions in the environment (US EPA 2010). Thus, HCN and the dissociated sodium and potassium cyanide salts are predominantly present as HCN in the stomach. HCN is rapidly absorbed by passive diffusion across cell membranes.

Cyanides do not accumulate in the blood or tissues following chronic or repeat exposure (WHO 2004, HPA 2011). In mammals, they are predominantly metabolised by the enzyme rhodanase to thiocyanates in the presence of sulphane-sulphur[14]. This conversion is irreversible and is the main metabolic detoxification pathway. While the liver is the primary organ for detoxification, all tissues contain rhodanase. The thiocyanate ion is readily excreted in urine. In rabbits, 80% of the formed

[13] Goitrogens are substances that can suppress the function of the thyroid gland and interfere with iodine uptake. In severe cases this may result in an enlargement of the thyroid, i.e. a goiter.

[14] This refers to one sulphur atom bonded to another sulphur atom such as in a thiosulphate salt (e.g. sodium thiosulphate).

thiocyanate is excreted within 24-48 hours; in dogs excretion is slower; and in sheep, 60% is excreted within 72 hours (FAO/WHO 1965).

RIVM (2000) indicates 47-89% of a dose of cyanide in the form of its metabolites is excreted in urine within 24 hours of ingestion, with small amounts also breathed out through the lungs (4% as HCN or CO_2). Cyanide can also be metabolised by lesser pathways including the complexation of cyanide with cobalt in hydroxocobalamin to form cyanocobalamin (vitamin B_{12}) or may be oxidised *in* vivo to carbon dioxide and formate and exhaled (HPA 2011, FAO/WHO 1965, WHO 2004, DEFRA 2002). The end products from these reactions are also excreted in urine. Figure 5.1 shows the primary metabolic pathways for cyanide. In all mammals, toxicity is the result of these detoxification pathways being overwhelmed by high exposures. The availability of sulphur from donor molecules is critical.

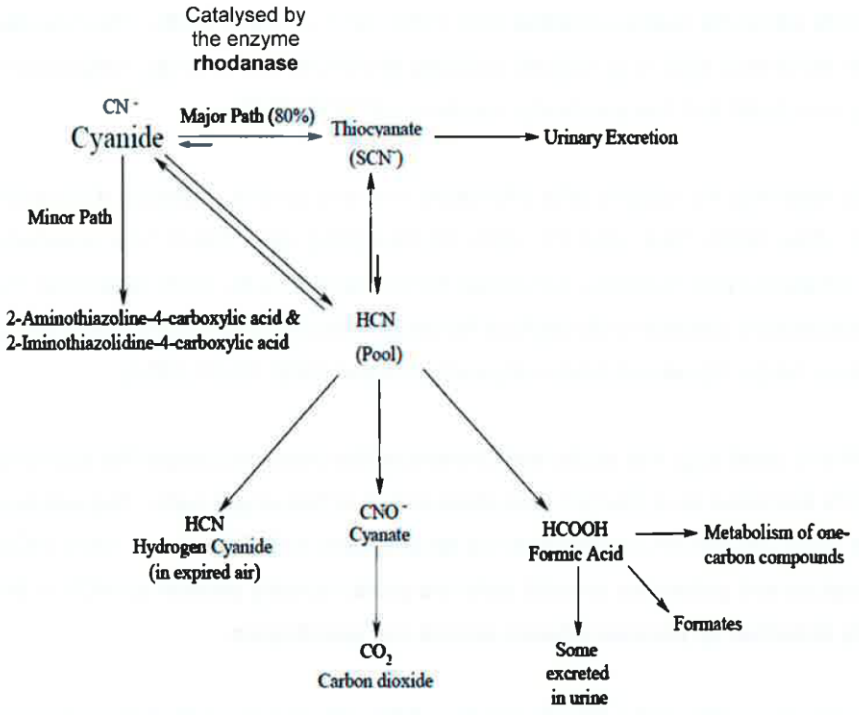


Figure 4.1: Primary metabolic pathways for cyanide
Source: US EPA 2010

Liver rhodanase activity is different in different species; rat>rabbit>human>dog (FAO/WHO 1965). However, the rate limiting factor for this detoxification pathway is not the amount of rhodanese enzyme itself, but availability of donor sulphur sources in the body (Gezondheidsraad 2002, FAO/WHO 1965). In humans the plasma half-life for cyanide transformation to thiocyanate is