Cyanides of Hydrogen, Sodium and Potassium, and Acetone Cyanohydrin (CAS No. 74-90-8, 143-33-9, 151-50-8 and 75-86-5)

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EXECUTIVE SUMMARY

This report has been produced as part of the ECETOC Joint Assessment of Commodity Chemicals (JACC) programme. It presents a critical evaluation of the toxicity and ecotoxicity data on hydrogen cyanide (HCN), sodium and potassium cyanides (NaCN and KCN) and acetone cyanohydrin (ACH). Most of these cyanides under physiological and environmental conditions will be present as HCN, which is the common toxic species (ACH dissociates into acetone and HCN). HCN (liquid or gas) and ACH (liquid) are used as chemical intermediates. NaCN and KCN (solids) are mainly used in silver and gold mining and in electroplating. All of these forms of cyanide are soluble in water.

Local releases of cyanide into water will be removed by volatilisation within a few days. Cyanide in water can exist as free HCN, or as complexes and salts, which may dissociate again to free HCN or adsorb onto sediment. Elimination of complex cyanides may take longer than free cyanide. In the global environment, cyanide is distributed to air and water. The main source of HCN in the atmosphere is the combustion of biomass. Airborne HCN undergoes slow photolysis, but the major part is absorbed into the oceans, where cyanide is removed by chemical and/or biological degradation. The overall atmospheric lifetime of HCN is 5 to 6 months.

Aquatic organisms (fish, invertebrates and algae) are very sensitive to cyanides. In the laboratory, concentrations as low as a few µg/l were found to be toxic. Water birds survived higher concentrations.

In mammals, cyanides (HCN) are very toxic regardless of the route of entry. HCN is rapidly absorbed and distributed within the body, and blocks the function of organs with great oxygen demand such as brain, heart and testes. Cyanides can also be lethal upon skin contact (even if only a small area is exposed) or following eye contact. There is no concern on the reproductive toxicity, and mutagenic or genotoxic activity of cyanides. No reliable carcinogenicity studies in animals exist (the quick onset of toxicity renders such studies impractical).

The dose rate is a critical factor in cyanide poisoning. The total amount of cyanide that can be tolerated is greater when the dose rate is below the detoxification rate. In long-term animal studies, where the dose is delivered over an extended period (such as in drinking water or in the diet) rather than in a bolus, no adverse effects were seen at levels markedly above the acute lethal dose.

Cyanide is normally detoxified in the body and eliminated as thiocyanate (in urine). Though less toxic than cyanide, chronic exposure to thiocyanate can exacerbate the effects of low iodine content in the diet and result in goitre. This is endemic in peoples who consume cassava as a

significant part of their diet, e.g. populations of Central Africa. Cassava is rich in cyanogenic glucosides.

Worker health studies with cyanides are inadequate for determining a no-adverse effect level in humans. Other studies (nutritional, epidemiological and clinical) indicate the absence of thiocyanate-mediated toxicity to the thyroid gland from daily doses equivalent to an occupational exposure (8-hour) of 7.5 mg CN^-/m^3 (time-weighted average) for humans with sufficient dietary iodine and normal kidney function. The acutely toxic cyanide concentration that can be tolerated by humans may be of the same order of magnitude. Sensitive sub-populations would include individuals with insufficient dietary iodine, insufficient thiosulphate supply (e.g. in the case of malnutrition) and impaired renal function. Tolerable exposure levels for cyanide salt dust may be lower (maximum 2 times, assuming 100% absorption).

Neurological disorders are known to occur in populations consuming food containing cyanogenic glucosides (e.g. cassava), particularly in combination with poor nutrition (when cyanide detoxification is impaired). There is no causal link between occupational exposure to cyanide and subjective symptoms of neurotoxicity. Parkinson-like symptoms have been reported in cases of acute cyanide poisoning (e.g. attempted suicide). Repeated low-dose exposure to cyanide has no such effects.

During the preparation of this document, draft versions were made available to SCOEL [a] and IPCS CICAD [b]. A hazard/risk assessment will be required under current OECD and proposed EU schemes [c,d].

[a] Scientific Commmittee on Occupational Exposure Limits of the EC
[b] International Programme on Chemical Safety, Concise International Chemical Assessment Documents
[c] OECD Existing Chemicals Programme
[d] EU Registration, Evaluation and Authorisation of Chemicals

THE ECETOC SCHEME FOR THE JOINT ASSESSMENT OF COMMODITY CHEMICALS

This report has been produced as part of the ECETOC Joint Assessment of Commodity Chemicals (JACC) programme for preparing critical reviews of the toxicology and ecotoxicology of selected existing industrial chemicals. In the programme, commodity chemicals (i.e. those produced in large tonnage by several companies and having widespread and multiple uses) are jointly reviewed by experts from a number of companies with knowledge of the chemicals. Only the chemical itself is considered in a JACC review; products in which it appears as a component or an impurity are not normally taken into account.

This document presents a critical evaluation of the toxicology, ecotoxicology, environmental fate and impact of the cyanides of hydrogen, sodium and potassium, and acetone cyanohydrin (CAS No. 74-90-8, 143-33-9, 151-50-8 and 75-86-5).

Special abbreviations, including symbols, units and prefixes, used in this report are explained in Appendix A.

Where relevant, the Task Force has graded the (eco)toxicological studies by means of a ‘code of reliability’ (CoR) (Appendix B) to reflect the degree of confidence that can be placed on the reported results.

1. SUMMARY AND CONCLUSIONS

This report reviews the available physico-chemical, ecotoxicity and toxicity data on hydrogen cyanide (HCN), sodium and potassium cyanides (NaCN and KCN) and acetone cyanohydrin (ACH). These substances are reviewed together because the cyanide anion (CN^-) or HCN is the common toxic species of the reviewed substances. As HCN is a relatively weak acid (dissociation constant Ka ≈ 10^{-9}), most of the cyanide under physiological and environmental conditions (pH ≈ 5 - 8) will be present as HCN.

Cyanide plays an important role in pre-biotic chemistry and the origin of life. Cyanide polymerisation and hydrolysis to formamide under specific conditions (cold environment where cyanide concentration is sufficiently high) is most probably responsible for the formation of the first nucleic and amino acids on earth (Miyakawa *et al*, 2002a,b). Cyanogenic molecules are found in a variety of organisms including many plants, e.g. apple seeds, peach pits, cassava.

HCN is a clear, volatile liquid or gas. It completely dissolves in water. NaCN and KCN are water soluble, white crystalline solid salts. ACH is a clear liquid that dissociates into acetone and HCN. ACH vapour decomposes rapidly into HCN and acetone. ACH in water undergoes rapid hydrolysis to HCN and acetone.

HCN, NaCN, KCN and ACH are produced in amounts greater than 100 kt per year worldwide. HCN is mainly used as an intermediate in the chemical industry for the production of chemicals such as adiponitrile, methyl methacrylate, cyanuric chloride or cyanide salts. The main areas of use for NaCN and KCN are gold and silver leaching in mining, electroplating, metal surface hardening and synthesis of organic chemicals. ACH is mainly used as an intermediate for the synthesis of methacrylic acid, methyl methacrylate, and other methacrylates.

When released into the environment, cyanide will distribute mainly to the atmosphere and water. The main source of HCN in the atmosphere is the combustion of biomass. Concentrations in the troposphere over the northern hemisphere amounted to 160 ppt by volume (≈ 180 ng/m^3). HCN will undergo photodegradation in the troposphere with a lifetime of one to several years (depending on the hydroxyl radical concentration), but the oceans act as a major and rapid sink for atmospheric HCN. This leads to an estimated overall atmospheric lifetime of 5.0 or 6.2 months. The oceanic sink is caused by a permanent under-saturation, probably due to chemical and/or biological degradation of cyanide. In the aquatic environment, cyanide can exist in numerous forms including free cyanide (HCN), cyanide complexes and less soluble cyanide salts. The fate and behaviour of the different cyanide species depends largely on their dissociation to free cyanide and their ability to adsorb onto sediment. Photolysis of cyanide complexes can liberate cyanide under the influence of sunlight. Volatilisation is expected to be an important and potentially dominant elimination process for cyanide released directly into water. Biodegradation,

complexation with subsequent adsorption onto sediment, and to a lesser extent photolysis and abiotic degradation, are additional possible routes of elimination. The half-life of free cyanide is mainly governed by volatilisation. It can be in the range of a few days to weeks depending on the wind speed and altitude, the temperature, the surface area, and the surface to volume ratio of the water body. The half-life of complex cyanides may be much longer.

Cyanide is acutely very toxic to aquatic organisms. Median lethal and/or effect concentration (LC_{50}/EC_{50}) values ranged from about 20 to 170 µg CN^-/l for fish, 2 to 1,000 µg/l for invertebrates and 45 to 500 µg/l for algae. Chronic no-observed effect concentration (NOEC) values were between < 1 and 5 µg CN^-/l for fish, 5 to 30 µg/l for invertebrates and 4 to 265 µg/l for algae. Probabilistic analysis of the large amount of available aquatic toxicity data led to the derivation of an acute tolerable concentration of 5 µg/l free cyanide and a long-term predicted no-effect concentration (PNEC) of 1 µg/l free cyanide for the aquatic environment.

For birds a tolerable concentration of 10 mg CN^-/l was derived.

In mammals, cyanide is rapidly absorbed in the form of HCN following oral, dermal or inhalation exposure. Cyanides are very toxic by all routes of entry. The mechanism of toxicity is by inhibition of oxygen utilisation by tissues; the most sensitive organs are those with the greatest oxygen demand such as brain, heart and testes. In humans, the acute lethal dose is approximately 1.5 mg/kgbw following oral uptake. The acute inhalation toxicity is a function of the body weight and the time of exposure. Using probit analysis of the acute inhalation data in different species, a human LC_{50} value of 202 mg/m^3 (180 ppm) and an LC_{01} of 88 mg/m^3 (78 ppm) were derived, both following 60 minutes of exposure. The lethal dose by the dermal route will depend upon the area of skin exposed. A dose of approximately 100 mg/kgbw is lethal even if only a small area of skin is contacted. Acutely toxic levels might also be achieved following eye contact.

Neurological disorders have been reported in populations consuming diets with high cyanogenic glucoside content (e.g. cassava). These populations are thought to have an increased sensitivity to cyanide due to poor nutrition and an associated impaired ability to detoxify cyanide. Occupational studies report a wide range of subjective symptoms suggestive of neurotoxicity that are not clearly related to cyanide exposure. A few published cases link cyanide exposure with Parkinson-like symptoms. These are all related to acute over-doses of cyanide (mostly in connection with attempted suicides) that were treated in a comatose stage. There is no indication from the available data that repeated low-dose exposure to cyanide could have similar effects.

The direct toxicity of cyanide prevents the conduct of studies on reproductive toxicity, and renders the available studies irrelevant. ACH showed no evidence of teratogenicity following gavage dosing of rats up to and including doses that produced maternal toxicity. In male and female fertility studies in rats, inhaled ACH showed no reproductive effects up to the onset of

acute toxicity (local irritant effects and systemic lethality), as indicated in repeated-dose inhalation studies. Therefore, although cyanide is overtly toxic to the reproductive system, there is no reason to suspect that this system is any more sensitive than are other organ systems.

Valid data are available for all genetic endpoints and there is no indication of mutagenic or genotoxic activity of cyanide. Although there are no reliable data on carcinogenicity in animals, the steep dose-response relationship would render the conduct of such a study impractical.

Cyanide is detoxified in the body through the formation and elimination in the urine of thiocyanate. Although less toxic than cyanide, thiocyanate can, in situations of chronic exposure (such as those occurring in cassava eating populations in Central Africa), exacerbate the effects of low iodine content in the diet resulting in commonly observed hyperthyroidism (goitre). Thiocyanate is a competitive inhibitor of iodide absorption by the thyroid gland and causes a decreased output of thyroxin by the thyroid. Re-adjustment to the original output of thyroxin requires an increase in thyroid mass (volume) brought about by increased secretion of thyroid stimulating hormone by the pituitary gland. Thyroid mass changes within 50% of normal are regarded as being within the physiological range with an increase of 500% or more being considered as goitre.

The toxicodynamics of cyanide poisoning in animals are complex and determined by a number of factors. These include the dose and rate of delivery of cyanide, the steep dose-response for acute toxicity, the rate and total capacity for detoxification (thiocyanate formation) and the rate of elimination of thiocyanate. In acute poisoning scenarios, the rate of delivery of cyanide can exceed the detoxification capacity resulting in overt acute toxicity. Whereas in chronic exposure scenarios, or where the rate of delivery is maintained within the detoxification capacity, this can be avoided. Consequently, it is possible to achieve a higher cumulative dose of cyanide when delivery is over a longer period than if it is delivered in a single bolus. This phenomenon is apparent in several of the mammalian toxicity studies reported with cyanides and is important in establishing a chronic no-observed adverse effect level (NOAEL) of relevance for humans. For example, in long-term dietary and drinking-water studies with cyanide in rats and mice, the NOAELs were 10.4 to 25.6 mg CN^-/kgbw/d. This is markedly above the median lethal dose (LD_{50}) of 3.3 to 3.9 mg CN^-/kgbw.

In 90-day guideline studies in rats administered cyanide via the drinking water (Hébert, 1993) or by inhalation (Monsanto, 1984), a NOAEL in the range of 10.4 to 12.5 mg CN^-/kgbw/d is indicated. This value is approaching, but below, the steep dose-response curve for acute lethality in this species and slightly below the observed NOAEL of 25.6 mg CN^-/kgbw/d in mice observed in a 90-day guideline drinking water study (Hébert, 1993). Lower NOAELs have been reported for dogs and miniature pigs but these studies are believed to be confounded by acute toxicity and

dietary complications. Generally, dogs tend to be more sensitive because of lower tissue levels of the enzyme rhodanese.

Available worker health studies involving occupational exposure to cyanides are too limited and lack essential detail regarding nutritional and iodine status. This renders them unreliable as a basis for setting a NOAEL for humans. Nutritional, epidemiological (morbidity) and clinical volunteer studies with cyanides, in contrast, typically include appropriate measurement of relevant clinical and dietary parameters such as dietary protein and iodide, serum and urinary thiocyanate, and smoking habits. These indicate the absence of adverse effects mediated via thiocyanate from daily doses equivalent to an 8-hour time-weighted average (TWA) exposure (e.g. occupational conditions) of 7.5 mg CN^-/m^3 for humans with sufficient dietary iodine and normal renal function. An analysis of the acute toxicity data and the human cyanide detoxification rates suggests that the tolerable concentration in humans with regard to cyanide-mediated acute toxicity may be of the same order of magnitude.

Sensitive sub-populations would include individuals with insufficient dietary iodine, insufficient thiosulphate supply (e.g. in the case of malnutrition) or impaired renal function. Iodine may need to be supplemented to restore and maintain the iodine-thiocyanate ratio (measured in urine, concentrations expressed in µg/mg) to above 3 to 4.

2. IDENTITY, PHYSICAL AND CHEMICAL PROPERTIES, ANALYTICAL METHODS

2.1 Identity

2.1.1 Hydrogen cyanide

Name:	Hydrogen cyanide
IUPAC name:	Hydrocyanic acid
Synonyms:	Cyclone
	Formonitrile
	HCN
	Prussic acid
Danish:	Blåsyre
Dutch:	Blauwzuur
Finnish:	Sinihapon, syaanivety
French:	Acide cyanhydrique
German:	Cyanwasserstoff
Greek:	Υδροκυανικό οξύ
Italian:	Acido cianidrico
Norwegian:	Blåsyre
Portuguese:	Acido cianidrico
Spanish:	Acido cianhidrico
Swedish:	Blåsyrans

CAS name:	Hydrocyanic acid
CAS registry No.:	74-90-8
Formula:	CHN
Molecular mass:	27.03
Structure:	H— C≡N

2.1.2 Sodium cyanide

Name:	Sodium cyanide
IUPAC name:	Sodium cyanide
Synonyms:	Cyanide of sodium
	Cyanogran
	Hydrocyanic acid, sodium salt
	Sodium salt of hydrocyanic acid
Danish:	Natriumcyanid

Dutch:	Natriumcyanide
Finnish:	Natriumsyanidi
French:	Cyanure de sodium
German:	Natriumcyanid
Greek:	Κυανιούχο νάτριο
Italian:	Cyanuro di Sodio
Norwegian:	Natriumcyanid
Portuguese:	Cianeto de sodio
Spanish:	Cianuro de sódio
Swedish:	Natriumcyanid

CAS name:	Sodium cyanide
CAS registry No.:	143-33-9
Formula:	NaCN
Molecular mass:	49.01
Structure:	Na—C≡N

2.1.3 Potassium cyanide

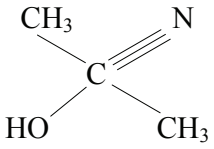
Name:	Potassium cyanide
IUPAC name:	Potassium cyanide
Synonyms:	Cyanide of potassium
	Hydrocyanic acid, potassium salt
	Potassium salt of hydrocyanic acid
Danish:	Kaliumcyanid
Dutch:	Kaliumcyanide
Finnish:	Kaliumsyanidi
French:	Cyanure de potassium
German:	Kaliumcyanid
Greek:	Κυανιούχο κάλιο
Italian:	Cyanuro di potassio
Norwegian:	Kaliumcyanid
Portuguese:	Cianeto de potasio
Spanish:	Cianuro de potasio
Swedish:	Kaliumcyanid

CAS name:	Potassium cyanide
CAS registry No:	151-50-8
Formula:	KCN

Molecular mass:	65.12
Structural formula:	K— C≡N

2.1.4 Acetone cyanohydrin

Name:	Acetone cyanohydrin
IUPAC name:	2-Hydroxy-2-methylpropionitrile
Synonyms	Acetoncyanohydrin
	2-Cyano-2-hydroxypropane
	2-Cyano-2-propanol
	2-Hydroxyisobutyronitrile
	2-Methyl-lactonitrile
	2-Propanone cyanohydrin
Danish:	Acetonecyanohydrin
Dutch:	Acetoncyaanhydrine
Finnish:	Asetonisyanohydriini
French:	Acétonecyanohydrine
German:	Acetoncyanhydrin
Greek:	Άκετονοκυανυδρίνη
Italian:	Acetoncianidrina
Norwegian:	Acetoncyanohydrin
Portuguese:	Acetona cianhidrina
Spanish:	Acetoncianhidrina
Swedish:	Acetoncyanhydrin

CAS name	Propane nitrile, 2-hydroxy-2-methyl-
CAS registry No.:	75-86-5
Formula:	C_4H_7NO
Molecular mass:	85.11
Structure:	

2.2 EU classification and labelling

HCN, NaCN, KCN and ACH are classified and labelled in accordance with the Dangerous Substances Directive 76/548/EEC and its subsequent amendments.

2.2.1 Hydrogen cyanide

EC (EINECS) No.:	200-821-6	
EC index No.:	006-006-00-X	
EC classification:	Extremely flammable, very toxic, dangerous for the environment	
EC labelling, symbol:	F+, T+, N	
Risk phrases:	R12	Extremely flammable
	R26	Very toxic by inhalation
	R50/53	Very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment
Safety phrases:	S7/9	Keep container tightly closed and in a well ventilated place
	S16	Keep away from sources of ignition - No smoking
	S36/37	Wear suitable protective clothing and gloves
	S38	In case of insufficient ventilation wear suitable respiratory equipment
	S45	In case of accident or if you feel unwell seek medical advice immediately (show the label where possible)
	S60	This material and its container must be disposed of as hazardous waste
	S61	Avoid release to the environment. Refer to special instructions/safety data sheet

2.2.2 Sodium and potassium cyanide

Sodium cyanide:
EC (EINECS) No.: 205-792-4

Potassium cyanide:
EC (EINECS) No.: 205-599-3

Sodium and potassium cyanide are not mentioned as such in Annex 1 of Directive 67/548/EEC. Listed are "hydrogen cyanide (salts of) with the exception of complex cyanides such as ferrocyanides, ferricyanides and mercuric oxycyanide".

EC index No.:	006-007-00-5	
EC classification:	Very toxic, dangerous for the environment	
EC labelling, symbol:	T+, N	
Risk phrases:	R26/27/28	Very toxic by inhalation, in contact with skin and if swallowed
	R32	Contact with acids liberates very toxic gas
	R50/53	Very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment
Safety phrases:	S7	Keep container tightly closed
	S28	After contact with skin wash with plenty of water
	S29	Do not empty into drains
	S45	In case of accident or if you feel unwell seek medical advice immediately (show the label where possible)
	S60	This material and its container must be disposed of as hazardous waste
	S61	Avoid release to the environment. Refer to special instructions/safety data sheet

2.2.3 Acetone cyanohydrin

EC (EINECS) No.:	200-909-4	
EC index No.:	608-004-00X	
EC classification:	Very toxic, dangerous for the environment	
EC labelling, symbol:	T+, N	
Risk phrases:	R26/27/28	Very toxic by inhalation, in contact with skin and if swallowed
	R50/53	Very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment
Safety phrases:	S7	Keep container tightly closed
	S27	Take off immediately all contaminated clothing
	S29	Do not empty into drains
	S45	In case of accident or if you feel unwell seek medical advice immediately (show the label where possible)

S60	This material and its container must be disposed of as hazardous waste
S61	Avoid release to the environment. Refer to special instructions/safety data sheet

2.3 Physical and chemical properties

2.3.1 Hydrogen cyanide

At normal (ambient) temperature and pressure, hydrogen cyanide (HCN) is a clear, almost colourless liquid or gas with a faint odour of bitter almonds. It is a weak acid that is completely soluble in water. Physical and chemical properties are listed in Table 1.

Table 1: Physical and chemical properties of HCN

Parameter, units	Value, unit HCN	Reference
Melting temperature	–13.24°C	Gail *et al*, 2000
	–13.4°C	US-EPA, 2000
Boiling temperature, at 1,013 hPa	25.70°C	Gail *et al*, 2000
	26°C	US-EPA, 2000
Relative density D_4^{20} (density of water at 4°C is 1,000 kg/m^3)	0.687	Gail *et al*, 2000
Viscosity at 20°C	0.192 mPa·s	Gail *et al*, 2000
Refractive index n_D at 20°C	No data	
Vapour pressure at 20°C	830 hPa	Gail *et al*, 2000
	807 hPa [a,b]	US-EPA, 2000
at 25°C	976 hPa [a,c]	US-EPA, 2000
Relative vapour density at 20°C (air = 1)	0.94	IPCS, 2003
Threshold odour concentration (air)	0.58 mg/m^3	ATSDR, 1998
Surface tension at 20°C	18.33 mN/m	Gail *et al*, 2000
Solubility in water at 20°C	No limit (miscible in all ratios)	Gail *et al*, 2000
Partition coefficient, log K_{ow} (octanol/water) at 20°C	–0.25 [d]	Hansch *et al*, 1995 cited by US-EPA, 2000
Partition coefficient, log K_{oc} (organic carbon/water) at 20°C	0.433 [a]	Meylan *et al*, 1992 cited by US-EPA, 2000
Henry's Law constant at 25°C	13.48 Pa·m^3/mol [d,e]	Gaffney *et al*, 1987
	0.00544 (air/water) [f]	
Flash point (closed cup)	–17.8°C	ATSDR, 1998; Gail *et al*, 2000
Explosion limits in air at 1,013 hPa at 20°C	5.5 - 46.5% (v/v)	Gail *et al*, 2000
pKa [g] value at 20°C	9.36	Izatt *et al*, 1962
at 25°C	9.21	
at 30°C	9.11	
Auto-flammability, ignition temperature	535°C	Gail *et al*, 2000

[a] Calculated
[b] Reported as 605 mm Hg (1 mm Hg = 1.33322 hPa)
[c] Reported as 732 mm Hg
[d] Measured
[e] Reported as 1.33×10^{-4} atm·m^3/mol
[f] Dimensionless ratio of concentration in air and water
[g] –log (acidity constant), measure of extent of acidity

Commercial forms of HCN are stabilised by the presence of typically 0.08 to 0.15% phosphoric acid by weight (0.8 - 1.5 g/kg) and 100 to 300 mg/kg sulphur dioxide (ICI Acrylics, 1997; Degussa, 2004).

2.3.2 Sodium and potassium cyanide

At normal (ambient) temperature and pressure, sodium cyanide (NaCN) and potassium cyanide (KCN) are hygroscopic white crystalline solids (salts) that are odourless when dry, but emit a slight odour of HCN and ammonia in damp air (Klenk *et al*, 1987; Gail *et al*, 2000). They are soluble in water. Physical and chemical properties are listed in Table 2 and 3.

Table 2: Physical and chemical properties of NaCN

Parameter, units	Value, unit NaCN	Reference
Melting temperature	561.7°C	Gail *et al*, 2000
Boiling temperature, at 1,013 hPa	1,500 ± 10°C	Gail *et al*, 2000
Relative density D_4^{20} (density of water at 4°C is 1,000 kg/m^3)	1.595	Gail *et al*, 2000
Viscosity at 20°C	No data	
Refractive index n_D at 20°C	No data	
Vapour pressure at 20°C	None	Gail *et al*, 2000
Vapour density at 20°C (air = 1)	Not applicable	
Threshold odour concentration (air)	Not applicable	
Surface tension at 20°C	No data	
Solubility in water at 20°C	370 g/kg	Gail *et al*, 2000
Partition coefficient, log K_{ow} (octanol/water) at 20°C	Not applicable	
Partition coefficient, log K_{oc} (organic carbon/water) at 20°C	No data	
Henry's Law constant at 25°C	Not applicable	
Flash point (closed cup)	Not applicable	
Explosion limits in air at 1,013 hPa at 20°C	Not applicable	
pKa value at 20°C	9.36	Izatt *et al*, 1962
at 25°C	9.21	
at 30°C	9.11	
Auto-flammability, ignition temperature	Not applicable	

Table 3: Physical and chemical properties of KCN

Parameter, units	Value, unit KCN	Reference
Melting temperature,	634.5°C	Gail *et al*, 2000
Boiling temperature, at 1,013 hPa	No data	
Relative density D_4^{20} (density of water at 4°C is 1,000 kg/m^3)	1.56 g/cm^3	Gail *et al*, 2000
Viscosity at 20°C	No data	
Refractive index n_D at 20°C	No data	
Vapour pressure at 20°C	None	Gail *et al*, 2000
Vapour density at 20°C (air = 1)	Not applicable	
Threshold odour concentration (air),	Not applicable	
Surface tension at 20°C	No data	
Solubility in water at 20°C	400 g/kg	Gail *et al*, 2000
Partition coefficient, log K_{ow} (octanol/water) at 20°C	Not applicable	
Partition coefficient, log K_{oc} (organic carbon/water) at 20°C	No data	
Henry's Law constant at 25°C	Not applicable	
Flash point (closed cup)	Not applicable	
Explosion limits in air at 1,013 hPa at 20°C	Not applicable	
pKa value at 20°C	9.36	Izatt *et al*, 1962
at 25°C	9.21	
at 30°C	9.11	
Auto-flammability, ignition temperature	Not applicable	

The commercial forms of NaCN and KCN are powder, granules and briquettes. The appearance of the powder and granules is irregularly formed grains. The briquettes are of a regular pillow shape.

2.3.4 Acetone cyanohydrin

At normal (ambient) temperature and pressure, acetone cyanohydrin (ACH) is a clear, almost colourless, liquid with a faint odour of almonds. It is completely miscible with water. Physical and chemical properties are listed in Table 4.

ACH dissociates to acetone and HCN, particularly when heated or in the presence of an alkali. Dissociation is reduced by addition of a small amount (0.1 - 0.15% by weight) of sulphuric acid (Ineos Acrylics, 2000a).

Table 4: Physical and chemical properties of ACH

Parameter, units	Value	Reference
Melting point	–19 - –20°C	Hartung, 1982
Boiling point	81 – 82°C [a]	NFPA, 1978; Verschueren, 1983
Decomposition temperature	120°C	NFPA, 1978; Verschueren, 1983
Relative density D_4^{20} (density of water at 4°C is 1,000 kg/m^3)	0.927 g/cm^3	Windholz, 1983
Viscosity at 20°C	Not applicable	
Refractive index n_D at 20°C	1.3992×10^{20}	Lide, 1991
Vapour pressure at 20°C	1.43 hPa [b]	Clayton and Clayton, 1982; Verschueren, 1983; Ineos Acrylics, 2000a
	4 hPa [c]	Jelpke, 2003; Grybat *et al*, 2003
Vapour density at 20°C (air = 1)	2.95	
Threshold odour concentration (air)	(Refer to HCN)	
Surface tension at 20°C	Not applicable	
Solubility in water at 20°C	Completely miscible	Windholz, 1983
Partition coefficient, log K_{ow} (octanol/water) at 20°C	–1 [d]	Rekker, 1977
Partition coefficient, log K_{oc} (organic carbon/water) at 20°C	1.04 [e]	Aster, 2003
Henry's Law constant at 25°C	1.01 Pa·m^3/mol [f]	Degussa, 2002a
Flash point (closed cup)	73 – 74°C	NFPA, 1978
pKa value	Not applicable	
Auto-flammability, ignition temperature	Not applicable	

[a] Measured at reduced pressure of 23 mm Hg (31 hPa) due to decomposition to HCN and acetone at 120°C and 172 mbar (169 hPa)
[b] Reported as 1.07 mm Hg
[c] Theoretical value for 'pure' ACH, see text; reported as 4 mbar
[d] Calculated
[e] Calculated following Lyman *et al*, 1990
[f] Calculated, hydrolysis (Section 4.3.2) not taken into account; reported as 1.03×10^{-5} atm·m^3/mol

Commercial ACH usually has a purity of between 98% and 99.5% ACH by weight, containing acetone (0.4 - 1.4%), HCN (0.02 - 0.1%, typically ≈ 0.05%) and water (0.05 - 0.6%) (all by weight). The relative amounts depend upon the source of nitrile (–C≡N) used (acrylonitrile or Andrussow HCN, Section 3.1.1). The impurities are all lighter than ACH and therefore contribute more to the vapour pressure than their concentration in the liquid would suggest. Also, ACH is continuously breaking down to HCN and acetone, this being suppressed, although not completely eliminated, by the addition of a stabilising acid (Jelpke, 2003).

The theoretical vapour pressure of 'pure' ACH (containing by weight: 98.95% ACH, 0.05% HCN, 0.5% acetone and 0.5% water) at equilibrium, can be predicted using the Aspen model as 4 hPa at 20°C (Grybat *et al*, 2003). This vapour consists of 33.55% HCN, 21.6% acetone, 13.2% water and 31.65% ACH by volume. In the case of commercial ACH, the actual vapour pressure will depend upon the extent of degradation of ACH to the more volatile HCN and acetone e.g. at 1.0% free acetone the vapour pressure at 20°C is 11.5 mbar and the vapour consists of 69% HCN, 15% acetone, 5% water and 11% ACH. The proportion of HCN is disproportionately high due to its greater volatility. Furthermore, ACH vapour is effectively not stabilised and will decompose rapidly to HCN and acetone (Jelpke, 2003).

Handbooks cite the vapour pressure of ACH as 1.4 hPa at 20°C (Clayton and Clayton, 1982; Verschueren, 1983). The difference reflects the fact that the observed vapour pressure is dependent on the purity of the ACH.

2.4 Conversion factors

Conversion factors for HCN concentrations in air at standard conditions (20°C and 1,013 hPa) are:

- 1 ppm = 1.124 mg/m^3
- 1 mg/m^3 = 0.890 ppm

Conversion factors for ACH concentrations in air at standard conditions (20°C and 1,013 hPa) are:

- 1 ppm = 3.538 mg/m^3
- 1 mg/m^3 = 0.283 ppm

Concentrations are expressed by volume as: ppm, parts per million (10^6); ppb, parts per billion (10^9) or ppt, parts per trillion (10^{12}).

Conversion factors for NaCN and KCN concentrations in air are not applicable.

In this report, converted values are given in parentheses. The generic formula, from which the conversion factors for vapour concentrations in air are derived, is given in Appendix C.

2.5 Analytical methods

2.5.1 Purity of cyanides

There is no method for the direct measurement of the purity of HCN. The HCN content is determined by subtraction of water and acid (stabiliser).

The cyanide content of alkali cyanides (solids and solutions) is determined by argentometry, i.e. the silver nitrate titration method developed by Von Liebig-Denigès, with potassium iodide as indicator (Denigès, 1898). The reaction equations are:

$$2\ CN^- + Ag^+ \rightarrow [Ag(CN)_2]^- \quad \text{(Eq. 1)}$$

$$I^- + Ag^+ \rightarrow \{AgI\}\downarrow \quad \text{(Eq. 2)}$$

Where 1 ml × 0.1 mol $AgNO_3$/l = 5.204 mg CN^- (equivalent to 9.802 mg NaCN or 13.022 mg KCN)

An accurate indication of the end-point of the titration is possible by potentiometry, using a combined Ag-calomel or Ag-thalamide electrode.

ACH is not routinely analysed for purity. The ACH content is estimated from the difference between the levels of total HCN and free HCN multiplied by a constant (3.149) (Robins, 2002).

2.5.2 Cyanides in air

HCN

The US-Agency for Toxic Substances and Disease Registry (ATSDR, 1998) reviewed measurement methods as follows. “HCN in environmental or workplace air is usually collected in sodium hydroxide solution and then measured spectrophotometrically after colour development (Agrawal *et al*, 1991; NIOSH 1989a). One of the most significant problems in cyanide monitoring is the instability of the collected samples (Cassinelli, 1986). The collection solution is pH > 11 to avoid volatilisation loss of molecular HCN. However, carbon dioxide from air may react with the solution during storage, thereby lowering the pH and releasing HCN gas. Oxidising agents in solution may transform cyanide during storage and handling. Ferrocyanide and ferricyanide complexes of cyanide undergo photodecomposition with ultraviolet light. Particulate cyanides are known to decompose in moist air with the liberation of HCN. The recommended method for the storage of cyanide samples is to collect the sample at pH 12 to 12.5 in closed, dark

bottles and store them in a cool, dark place. It is also recommended that the samples be analysed immediately upon collection. The sample handling and preservation methods have been discussed (Cassinelli, 1986; Egekeze and Oehme, 1979). Cyanide determination in air usually distinguishes between two forms of cyanides: HCN gas and particulate cyanides. Filters are usually used to collect particulate cyanides, and the HCN gas that passes through the membrane is trapped in sodium hydroxide. The collected particulate cyanides can be quantified separately after acid distillation. Detection limits are in the ppm range for occupational air (Dolzine *et al*, 1982; NIOSH, 1989a,b) and sub-ppm range for ambient air (Cassinelli, 1986). Reported recovery is good (> 90%) (Cassinelli, 1986; Dolzine *et al*, 1982; NIOSH 1989a,b)" (All references cited by ATSDR, 1998).

Measurement of HCN in the ppm range is possible with Dräger test tubes or with portable or stationary HCN detectors from Dräger or Monitox. The basis of these detectors is an electrochemical cell, which allows a continuous monitoring of HCN in the air (Drägerwerk, 1980; Bayer Diagnostic and Electronic, 1986).

Another method starts with absorption of airborne HCN into a sodium hydroxide solution. This solution is then analysed following standard DIN/ISO [a] and ASTM [b] methods for NaCN and KCN (Section 2.5.3).

Detection of HCN in the ppb range is also possible using gas chromatography (GC) with infra-red detection (Visser, 2000).

HCN is detectable in the atmosphere in the ppt range using spacecraft-based ion-molecule reaction mass spectrometry (Reiner *et al*, 1998) or Fourier transform spectrometry (e.g. Rinsland *et al*, 2000).

NaCN and KCN

For the measurement of airborne NaCN and KCN dust, the air is passed through a fine filter paper. The captured dust is dissolved in a caustic soda solution and titrated against a 0.01 mol/l $AgNO_3$ solution (Section 2.5.1).

[a] Deutsches Institut für Normung/International Organization for Standardization

[b] American Society for Testing and Materials

ACH

No routinely used method exists for the measurement of ACH in air. Due to the rapid dissociation of ACH to HCN and acetone, the recommended method for determination of airborne ACH is to measure atmospheric HCN levels as these will always predominate.

2.5.3 Cyanides in water, soil, sediments

The cyanide content of solid alkali metal cyanides, alkaline earth metal cyanides, and dissociating heavy metal cyano-complexes such as $[Zn(CN)_4]^{2-}$ in environmental media may be determined by argentometry, using a dilute aqueous solution and preferably the potentiometric endpoint (Section 2.5.1). This method is referred to as the determination of free cyanide.

For more stable cyano complexes or insoluble cyanide compounds such as iron(II) ferrocyanide, $Fe_2[Fe(CN)_6]$, or in the presence of substances that disturb the argentometric titration, the cyanide must be liberated and separated before the actual analysis. This can be done by distillation, i.e. acidifying and boiling the cyanide solution or suspension, and absorbing the released HCN in sodium hydroxide solution. Depending on the concentration, the cyanide content of the absorption solution may be analysed argentometrically or colorimetrically, e.g. by using pyridine-barbituric acid reagents (Asmus and Garschagen, 1953). This method is called the determination of total cyanide, because practically all cyanides (except extremely stable cyanide complexes, such as that of cobalt) can be liberated for determination. There is an ISO standard for total cyanide determination (ISO 6703/1) (DIN, 1981). The ASTM determination method for total cyanide is also used internationally (ASTM, 1982).

Another ISO standard for the 'Determination of easily liberated cyanide' (ISO 6703/2) is a reliable method for measuring less stable complexes of cyanide, such as those occurring in wastewaters and effluents that contain cyanide. HCN, alkali and alkaline earth metal cyanides, and the cyanides of zinc, cadmium, silver, copper, and nickel can be determined by this method. The method is not suitable for complex cyanides of iron, cobalt, gold, and nitriles. Following the ISO 6703/2 method, HCN is liberated (distilled off) at pH 4 in the presence of zinc metal and ethylenediamine tetra-acetic acid. The HCN is transferred to an absorption vessel and analysed argentometrically or colorimetrically (DIN, 1981). The ASTM method for the determination of less stable complexes of cyanide is called the weak acid dissociable (WAD) cyanide method (ASTM, 1982).

Another method for cyanide analysis, similar to the above two ISO/ASTM distillation methods, is the colorimetric picric acid method (Barkley and Ingles, 1970). This method has gained in importance over the last decade, since it yields results comparable to the two standard methods

but does so more rapidly. The speed is crucial where the effluent is discharged continuously, e.g. from a gold mine.

Other methods for the determination of cyanide such as colorimetry with pyridine-barbituric acid reagents (Asmus and Garschagen, 1953) and use of ion-selective electrodes (Smith and Mudder, 1991) for free cyanide, are prone to interference by various substances. The ion-chromatographic method has been a subject of research in recent years, and techniques have been developed to determine several cyanide species selectively, especially at low concentrations. However, these techniques and instrumentation have not yet achieved widespread application (Smith and Mudder, 1991).

Quick tests to check the concentration of cyanides in wastewater are based on various colorimetric methods (Asmus and Garschagen, 1953; Barkley and Ingles, 1970). Generally, the reactions permit detection of very small amounts of free cyanide, but they can be disturbed by various substances, e.g. reducing agents or thiocyanate (SCN^-), sulphide, and nitrite, which all produce similar colours.

2.5.4 Cyanides in biological media

Gettler and Baine (1938) evaluated various methods for the measurement of cyanides in biological media. The tissue samples (humans and dogs) and processing equipment were refrigerated to prevent any prior loss of HCN by volatilisation during storage and grinding. Various methods were discussed for preliminary (qualitative) detection of HCN, e.g. by means of copper sulphate-guaiac filter paper placed for 0.5 hours over the finely ground and acidified tissue sample. A blue colour on the paper would indicate cyanide poisoning, to be confirmed by other, less rapid and/or more sensitive tests (described in the paper). For quantitative determination, the authors isolated and recovered cyanides (as HCN) from homogenised tissues by gentle distillation in the presence of tartaric acid. The tissue distillate (HCN vapour) was absorbed into sodium hydroxide (NaOH) solution and aliquots of the alkaline solution analysed for HCN by argentometry (Section 2.5.1). The endpoint (formation of silver iodide, AgI) was sufficiently sharp to allow for accurate determination of 0.03 to 2 mg HCN/25 ml; below 0.03 mg the method gave slightly high results. Another accurate method in the range 0.5 to 10 mg HCN/50 ml was colorimetry of ferric thiocyanate, $Fe(SCN)_3$, formed by addition of polysulphide (ammonium, NH_4OH, solution saturated with H_2S) and iron chloride ($FeCl_3$) solutions. Other methods such as iodometric titration, colorimetry of Prussian blue and gravimetry of silver cyanide (AgCN) were found to be unsuitable for the determination of small quantities of HCN, especially in the presence of other volatile organic matter.

Cyanide analysis in biological media is of interest for estimation of the body burden of cyanide after accidental or dietary exposure of humans and in experimental exposure of laboratory animals. Attention should be paid to selecting the appropriate tissue or body fluid, time of sampling, storage conditions and time to analysis.

In the case of death caused by acute poisoning, the preferred order of analysis is blood, lung, myocardium, brain and in humans also the spleen. The choice of these tissues is based on the finding of consistent and moderately high concentrations of cyanide in them following death from acute cyanide exposure by all routes of administration (Troup and Ballantyne, 1987).

Time of sampling is critical because cyanide levels decrease by a variety of reactions in tissues and body fluids. Following acute poisoning, cyanide could not be measured 3 days *post mortem* in the liver or kidney, 14 days *post mortem* in brain or lung, and 3 weeks *post mortem* in blood. Similar observations were made in tissues removed from the body immediately after death. Cyanide could not be detected after 3 days in kidney or liver, 7 days in brain and 14 days in lung. However, significant levels could still be measured in whole blood after 3 weeks (Ballantyne, 1974 cited by Troup and Ballantyne, 1987).

The basis of the analytical method is the isolation of cyanide from the tissue and its concentration in a clean solution. Generally, the cyanide is liberated from the biological specimens by treatment with an acidic solution and converted into HCN, which is carried over into an alkaline solution in order to trap the cyanide as non-volatile NaCN. HCN may be transferred from the acidification vessel to the alkaline absorption by simple (static) vapour diffusion or, dynamically, by passing an air or nitrogen stream through the acid solution and then bubbling the resultant HCN-containing gas through the absorption solution (Troup and Ballantyne, 1987).

The cyanide in the absorption solution is measured by colorimetry or fluorometry, with a cyanide ion specific electrode or by GC. In newer methods, the absorption step has been omitted. The sample is acidified with phosphoric acid and the air of the headspace sampled and analysed by GC with nitrogen detection (Calafat and Stanfill, 2002).

A gas-phase electrochemical technique has been proposed for detecting and measuring HCN in biological tissues. The method involves freeing a sample of cyanide gas (or carbon monoxide) from the material examined and introducing it into a gas-phase electrochemical detection system. Automation of the technique would allow rapid analysis (a few minutes/sample), which is an advantage in accident and product-tampering (*post mortem*) investigations. No specific details were given in the research summary (Logan, 1996).

Tracqui *et al* (2002) reported a sensitive and specific combination of high performance liquid chromatography and mass spectrometry (HPLC-MS) method for the determination of cyanide in

whole blood. Radio-labelled $K^{13}C^{15}N$ was added as an internal standard to blood samples that were then placed in a micro-diffusion device. The inner well of this micro-diffusion cell was filled with a mixture of taurine, naphthalene-2,3-dicarboxaldehyde, methanol and ammonia solution. Concentrated sulphuric acid was added to the blood sample and the vial was sealed immediately. Free HCN diffused to the inner vial where cyanide formed a fluorescent 1-cyano-benzisoindole derivative with naphthalene-2,3-dicarboxaldehyde-taurine that was analysed by HPLC using a C18 column, a gradient of acetonitrile in ammonium acetate (2 mmol/l) pH 3 buffer (35 to 80% in 10 min) and a fluorimetric detector as well as MS. The limit of detection was 5 ng CN^-/ml and the limit of quantification 15 ng/ml.

In the case of chronic low exposure to cyanide, the daily dose can be better estimated by measurement of thiocyanate (SCN^-), the main metabolite of cyanide, in plasma or urine. The half-life of thiocyanate in plasma is 2.7 days (Schulz, 1984). This means that measurement of thiocyanate in urine reflects the cyanide intake over a period of a few days. To determine thiocyanate in plasma, the proteins were precipitated with trichloroacetic acid leaving the thiocyanate in the supernatant. An alternative was to adsorb thiocyanate on a weak anion-exchange resin and elute with perchlorate. The thiocyanate in the supernatant or eluate was analysed by direct colorimetry using ferric ions, Fe^{3+} (Aubry *et al*, 1988; Heuse *et al*, 1989).

Lundquist *et al* (1995) preferred chlorination of thiocyanate with hypochlorite to cyanogen chloride followed by a colorimetric reaction with isonicotinic acid and 1,3-dimethylbarbituric acid. The detection limit was 0.93 µmol/l.

Earlier methods used potassium permanganate to oxidise thiocyanate to cyanide and then determine the resulting cyanide concentration. In the case of high cyanide levels in blood, it was necessary to remove cyanide before the oxidation of thiocyanate. This was done by bubbling oxygen-free nitrogen through the acidified solution (Maehly and Swensson, 1970; Ballantyne, 1977).

BASF (2001) developed a method to determine urinary thiocyanate. Urine samples were shaken for 2 hours at 30°C following addition of potassium hydroxide (2 mmol KOH/l), an internal thiocyanate standard and a derivation solution (pentafluorobenzyl bromide in dichloromethane). Aliquots of 1 µl were then injected into a GC equipped with a capillary column and mass selective detector. The detection limit was 0.5 mg SCN^-/l urine.

Tsuge *et al* (2000) combined head space GC for cyanide and the modified colorimetric thiocyanate assay for simultaneous determination of cyanide and thiocyanate in blood or saliva. Ascorbic acid was used in the head space GC to prevent conversion of thiocyanate to cyanide, which is a confounding factor in conventional acidification procedures.

2.6. Summary and evaluation

Classical titration methods such as argentometry with colorimetric or electrochemical detection are still of relevance for the determination of purity and high concentrations of solid and liquid NaCN and KCN. The purity and content of HCN and ACH is assessed indirectly.

HCN in air is frequently sampled by collection in sodium hydroxide solution. Cyanides in particulate form are collected on filter materials. Subsequent analysis can be performed by a number of methods depending on the concentration.

Direct analysis of workplace air can be performed by electrochemical methods (HCN gas detectors) or colorimetric methods (HCN test tubes). GC-IR, GC-MS, HPLC-MS or head space GC with different detectors are suitable to detect and quantify HCN concentrations in the ppb range.

Traces of free cyanide ions can be determined by conventional silver nitrate titration or colorimetric determination (barbituric acid pyridine method).

For the determination of weak complex cyanides and total cyanide, a number of standard methods are available (ISO, DIN, ASTM), that are preferably used for the analysis of water. Easily liberated cyanides (weak complex cyanides) can also be determined by colorimetry using picric acid.

Determination of cyanide in biological media normally involves liberation of HCN (acidification and separation by distillation or micro-diffusion) and subsequent analysis. Historically, this has been done by colorimetric or fluorometric methods after derivatisation. Newer, more reliable methods include head space GC or HPLC-MS. For the determination of cyanide in biological media, sample preparation and storage are critical. This is because HCN may volatilise from the samples or additional cyanide may be liberated from thiocyanate that is generally present in higher concentrations than cyanide itself. For biomonitoring purposes, thiocyanate determination in blood or urine, or a combination of cyanide and thiocyanate analyses, is considered the method of choice.

3. PRODUCTION STORAGE, TRANSPORT AND USE

3.1 Production

3.1.1 Hydrogen cyanide

HCN is produced by direct reaction of alkanes with ammonia, and indirectly as a by-product of the manufacture of acrylonitrile (by ammoxidation of propylene to acrylonitrile). The various processes are described below.

Mudder and Botz (2001) reported that 1,400 kt of HCN were produced annually, of which 13% was converted into NaCN for use in mining, which is the main application of NaCN. This would correspond to approximately 330 kt/y of NaCN. There are also other consumers of NaCN such as the chemical industry and the surface treatment industries.

In 2002, the total world-wide HCN capacity was 2,165 kt, including 677 kt as a by-product of acrylonitrile manufacture and 1,488 kt 'on-purpose' (PCI, 2002).

Gail *et al* (2000) reviewed the Andrussow, methane-ammonia and Shawinigan processes for HCN manufacture as follows. (Primary references including several patents have been omitted for the purpose of this report.)

Andrussow process

The Andrussow process was developed around 1930 and it is currently the most widely used method for direct synthesis of HCN. The average capacity of single commercial installations is 5 to 30 kt/y; large plants are operated in Western Europe, USA and Japan.

Natural gas, essentially sulphur-free methane, is mixed with ammonia. Compressed air is added in a volume ratio that corresponds closely to the theoretical reaction:

$$CH_4 + NH_3 + 1.5\ O_2 \rightarrow HCN + 3\ H_2O \quad \text{..... (Eq. 3)}$$

Where the enthalpy change DH = – 474 kJ/mol

The mixture is passed over a platinum-rhodium or platinum-iridium gauze catalyst. The temperature and upper flammability limit are monitored carefully. The exothermic reaction takes place at high temperature (> 1,000°C) and normal atmospheric pressure; the gas velocity through

the catalyst zone is 3 m/s. To avoid decomposition of HCN, the effluent gas from the reactor is quickly cooled in a waste-heat boiler, which produces steam used further on in the process.

After passing through the waste-heat boiler, the gas is washed with dilute sulphuric acid to remove unreacted ammonia. This is necessary to prevent polymerisation of HCN. Because disposal of the resulting ammonium sulphate solution is expensive, other systems have been patented. Alternatively, the off-gas from the reactor is passed through a mono-ammonium phosphate solution in an absorber. This converts the ammonia to diammonium phosphate. To effect thermal reversal of the phosphate equilibrium, the absorption solution is transferred into a stripper and boiled by injection of steam. The released ammonia is condensed and recycled to the reactor, while the regenerated mono-ammonium phosphate solution is pumped back to the absorber.

After the ammonia scrubber, the gas is passed through a counter-current column in which the HCN is absorbed in cold water. The resulting solution is stabilised by adding acid (≈ 0.1%). The HCN is stripped from the aqueous solution in a rectifier and condensed. The end product is of high purity and has a water content of less than 0.5%. The aqueous absorber solution, containing traces of HCN, is cooled and fed back to the absorption tower. The residual gases (H_2, CO, and N_2) can be used for heating or be methanated in a separate unit and recycled as feedstock for HCN manufacture.

The advantages of the Andrussow process include: (i) a long catalyst life of up to 10,000 hours, (ii) a well-tested technology in a simple and safe reaction system, and (iii) the yield of high-purity HCN. A disadvantage is that the process is dependent on pure methane as a raw material to avoid carburisation of the platinum catalyst. Even a small percentage of higher hydrocarbon impurities will rapidly affect the platinum-iridium catalyst and reduce conversion rates. Other important ‘poisons’ of platinum are sulphur and phosphorus compounds. A further problem of the Andrussow process is the relatively low HCN yield (based on gas with 60 to 70% methane and 70% ammonia content, by volume) and the low HCN concentration in the product gas, so that recovery equipment must be capable of handling large volumes of gas.

Methane-ammonia process

The basis of the methane-ammonia or ‘BMA’ [a] process, developed by Degussa, is the formation of HCN in the absence of oxygen.

[a] Blausäure-Methan-Ammoniak

$$CH_4 + NH_3 \rightarrow HCN + 3\ H_2 \quad \text{..... (Eq. 4)}$$

Where the enthalpy change D*H* = + 252 kJ/mol

The reaction is endothermic and requires temperatures above 1,200°C. It is performed in externally heated tube bundles made of alumina (aluminium oxide) coated with a thin layer of a special platinum catalyst. Several of these bundles are fixed in a reaction furnace unit. A mixture of ammonia and methane (natural or refined gas with a methane content of 50 to 100% by volume) is passed through the tubes and quickly heated to 1,300°C at normal atmospheric pressure. To avoid the formation of any disturbing deposits of carbon black, the NH_3/CH_4 ratio is kept between 1.01 and 1.08. After leaving the reaction tubes, the product gas is cooled to 300°C by passage through a water-cooled aluminium chamber. A kinetic study has shown that a particular temperature profile is essential for this process.

The subsequent reaction steps, ammonia absorption and HCN isolation, are similar to those of the Andrussow process. A distinct advantage of the methane-ammonia process is the higher HCN content of the product gas, thereby reducing the number of purification steps and the size and cost of recovery equipment. The tail gas consists mainly of pure hydrogen. If this is not needed for other syntheses, it can be used as fuel for heating the furnace. About 80 to 87% of the ammonia and 90 to 94% of the methane are converted to HCN. The specific energy consumption of approximately 4 GJ/100 kg HCN reported previously has been considerably decreased by more recent developments. A large part of the heating energy is recovered and used in the air pre-heater or steam generator.

If the methane supply is limited, the process can be carried out directly with liquefied hydrocarbons or ethanol, or in a three-step reaction starting from methanol. Both Degussa and Lonza utilise the methane-ammonia route to produce HCN.

Shawinigan process

In the Shawinigan process, developed in 1960 by Shawinigan Chemicals [a], hydrocarbon gases are reacted with ammonia in an electrically heated, fluidised bed of coke. The process is also known as the Fluohmic process.

In a circular reaction cavity constructed from alumina and silicon carbide, a mixture of ammonia and hydrocarbon (N/C ratio slightly > 1) is passed through a fluidised bed of coke, heated by

[a] Now a division of Gulf Oil Canada

electrodes immersed in the bed. The chemical reaction is similar to the methane-ammonia process, but no catalyst is required and temperatures are kept above 1,500°C. Propane is usually the main feedstock. Other carbon compounds, such as naphtha or lighter hydrocarbons, can also be converted. The endothermic reaction can be described as:

$$3\ NH_3 + C_3H_8 \rightarrow 3\ HCN + 7\ H_2 \quad \text{(Eq. 5)}$$

Where the enthalpy change DH = + 634 kJ/mol

In practice, at least 85% of the ammonia and up to 94% of the hydrocarbon are converted to HCN. Unreacted feed gas is almost completely decomposed to the elements. This reduces the quantity of ammonia to be removed from the product gas and leads to the formation of coke particles. The control of coke particle size in the bed is an important operating parameter.

The reactor effluent gas contains, by volume, up to 25% HCN, 72% H_2, 3% N_2 and 0.005% NH_3. Coke is removed in a water-cooled, cyclone-entrained bed. The gas is further cooled and enters the absorption equipment where HCN is removed. The residual gas, nearly pure hydrogen, can be used for other chemical processes. Some of the hydrogen is recycled to the reaction unit to inhibit the formation of soot. Coke from the cyclone is screened, and three fractions are separated, stored, and then fed back to the reactor system in the desired proportions to control the particle size distribution. By regulating the rate of coke recycling, the level of the fluidised bed and the reaction temperature can be controlled.

Because of the high electric power consumption (6.5 kWh/kg HCN) the Shawinigan process is attractive where low-cost electricity is available. This process is employed by Polifin in South Africa, Arogenesas in Spain and Ticor in Australia.

By-product of acrylonitrile

HCN is currently also produced indirectly, as a by-product of the manufacture of acrylonitrile, by ammoxidation of propylene. Propylene is reacted with ammonia and oxygen (or air) in a fluid bed catalyst at a temperature of about 450ºC. This leads to exothermic formation of acrylonitrile.

$$2\ C_3H_6 + 2\ NH_3 + 3\ O_2 \rightarrow 2\ CH_2(\text{–}CH)\text{–}CN + 6\ H_2O \quad \text{(Eq. 6)}$$

Simultaneously, HCN is generated in an exothermic reaction, following:

$$2\ C_3H_6 + 2\ NH_3 + 8\ O_2 \rightarrow 2\ HCN + 8\ H_2O + 4\ CO_2 \quad \text{(Eq. 7)}$$

The ratio of HCN to acrylonitrile produced depends mainly on the type of (fluid bed) catalyst used in the process and may vary by more than 10% by volume. The output of HCN may be 'boosted' by injection of methanol into the reactor.

3.1.2 Sodium and potassium cyanide

Gail *et al* (2000) reviewed the main processes for NaCN and KCN production as follows. (Primary references including several patents have been omitted for the purpose of this report.)

The large-scale production of alkali metal cyanides began in the second half of the 19th century, when J. S. Macarthur and the Forrest Brothers patented their process for the extraction of gold and silver from ores. The manufacturing method was based on work of F. and E. Rodgers, who fused potassium or sodium carbonate (K_2CO_3, Na_2CO_3) with potassium ferrocyanide ($K_4[Fe(CN)_6]$, from animal blood and waste). Although a large number of modified processes were patented up to 1900, industrial production of cyanides began in earnest following the two-stage process of H. J. Castner. This was further developed mainly at Degussa and used there until 1971. The Castner process employed the reaction of sodium metal with charcoal and ammonia:

$$2\ Na + 2\ C + 2\ NH_3 \rightarrow 2\ NaCN + 3\ H_2 \quad \text{(Eq. 8)}$$

Today, NaCN and KCN are produced exclusively by neutralisation of a solution of sodium or potassium hydroxide with liquid or gaseous HCN. The stoichiometric reaction is:

$$NaOH + HCN \rightarrow NaCN + H_2O \quad \text{(Eq. 9)}$$

Although the process looks very simple, it is fraught with a number of technical difficulties. These result from the tendency of HCN to polymerise, the hydrolysis of the alkali metal cyanides and their reactivity with carbon dioxide and air. High-grade products can be obtained only when the production process is controlled very closely. In principle, all production plants employ the same process steps, which may differ in detail, as follows.

Gaseous or liquid HCN is first introduced continuously into concentrated solutions of NaOH or KOH at elevated temperature. The heat of neutralisation is used for the subsequent vaporisation of water. The use of special mixing devices and the addition of 0.2 to 3% excess NaOH prevents polymerisation. Simultaneously, or in a second step, water is vaporised under reduced pressure and the alkali metal cyanide is precipitated below 100°C. The shorter the residence time of the cyanide in solution, the cleaner is the product and the lower the content of hydrolysis products and iron impurities.

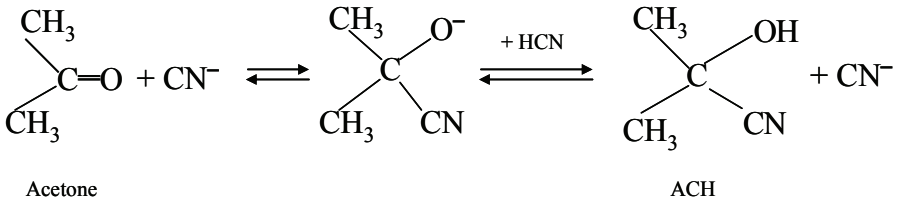
The cyanide crystals are separated by filtration or centrifugation, and various methods are used to dry the wet salt, which contains about 12% water. A two-step drying process using hot, CO_2-free air at 200 to 430°C is advantageous because the product remains free-flowing. Sometimes fluidised-bed dryers are utilised. In any case, the used air must be washed and the wastewater detoxified to avoid environmental pollution. Standard equipment is used for further processing of the NaCN or KCN salt, which still contains up to 1% moisture at this stage. The final commercial forms are powder, granules, briquettes or tablets.

3.1.3 Acetone cyanohydrin

Large-scale production of ACH began in the 1930s as an intermediate in the production of methyl methacrylate from HCN.

ACH is manufactured by the base-catalysed condensation of acetone with HCN according to the following mechanism (Sifniades, 1987) (Figure 1).

Figure 1: Reaction of acetone with HCN, yielding ACH (Sifniades, 1987)



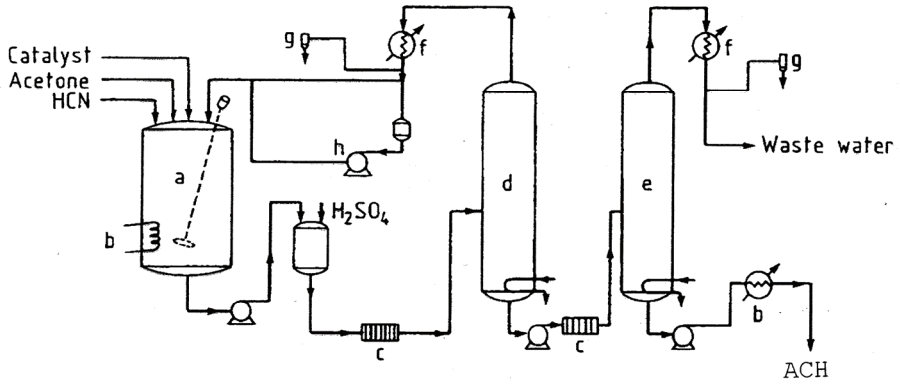
Acetone ACH

The reaction is reversible but formation of the cyanohydrin is quite favourable; the equilibrium constant is 28 l/mol at 20 to 25°C. Following an isothermal reaction stage at approximately 40°C, time-delayed chilling to 0°C is used to drive the reaction to approximately 90% completion. The reaction is usually carried out in the liquid phase. Representative catalysts used industrially are sodium hydroxide, potassium hydroxide, potassium carbonate, and anion exchange resins (Sifniades, 1987).

A schematic flow sheet of the Rohm and Haas process is shown in Figure 2. Acetone and liquid HCN are fed continuously to a cooled reactor along with an alkaline catalyst. The catalyst is then neutralised with sulphuric acid and the resulting salt is removed by filtration. The crude product is then distilled in a two-stage process. The overheads from the first column consist mainly of acetone and HCN, which are recycled to the reactor. The second column removes water overhead and leaves ≥ 98% pure ACH at the bottom. The manufacture of ACH produces no by-products other than small amounts of sulphate salts formed during catalyst neutralisation. The ACH

product is cooled and stored prior to being used for manufacture of methyl methacrylate (Sifniades, 1987).

Figure 2: The Rohm and Haas ACH process (Sifniades, 1987)



[a] Reactor
[b] Cooling
[c] Filter press
[d,e] Concentrator
[f] Condenser
[g] Vacuum jet
[h] Pump for acetone and HCN recycle

3.2 Storage

3.2.1 Hydrogen cyanide

Handling, storage and transport of HCN are determined by its low boiling point, high toxicity, and instability in the presence of moisture, bases, or other impurities. The liquid acid is relatively non-corrosive. Materials compatible with HCN at normal temperatures are stainless steel, Hastelloy and Monel. To prevent polymerisation, stabilising agents such as sulphuric acid, phosphoric acid, oxalic or acetic acid, and sulphur dioxide are used. The type and quantity of stabiliser (usually < 0.5% by weight) depend on storage capacity, temperature, and residence time in a container. A combination of H_2SO_4 and SO_2 prevents decomposition of HCN in the liquid and vapour phases. Larger quantities of HCN are stored at a maximum temperature of 5°C and must be permanently re-circulated.

Additionally, the colour of the liquid is monitored and should not exceed American Public Health Association (APHA) colour number 20 (visual yellowness compared to a reference value of 100 for a standard platinum-cobalt solution), to avoid polymerisation. To keep the concentration of

gas below the danger level, good ventilation of buildings in which HCN is stored and handled is of primary importance.

3.2.2 Sodium and potassium cyanide

Any type of packaging that contains NaCN or KCN is stored in a dry place and protected against corrosion. Rooms in which cyanides are stored and processed are well ventilated. A full mask with a type B filter (identifying colour grey, shelf-life unused ≤ 5 years) must be worn when HCN might be present. The cyanide storage area is clearly marked and unauthorised persons are never allowed access to rooms in which cyanides are stored or processed. Therefore, a cyanide area is normally separated from other areas, e.g. by fences or walls. In case of an emergency, powder fire extinguishers, safety showers, eye washes, and antidotes should be available in the immediate vicinity.

If the original cyanide drums are used as storage containers, they must be tightly closed immediately after removal of cyanide and should not be allowed to stand open. Stainless steel has been used as construction material for equipment that comes in contact with cyanide solutions up to 100 t. Concentrated cyanide solutions are stored in steel or stainless steel containers protected against leakage by a collecting basin constructed of alkali-resistant concrete (CEFIC, 2006).

3.2.3 Acetone cyanohydrin

Acid stabilised ACH is stored under ambient conditions. No further addition of acid is required during storage. Storage vessels of up to several thousand tonnes capacity are common. ACH is relatively non-corrosive and can be stored in carbon steel or stainless steel tanks. The vapour pressure of ACH is such that a flammable mixture does not exist at ambient temperature; therefore storage under inert gas is not necessary.

The ACH storage area must be clearly marked and unauthorised persons should not be allowed access within the confines of the bunded area. In case of an emergency, safety showers, eyewashes, and antidotes should be available in the immediate vicinity. Atmospheric HCN detection is normally provided.

3.3 Transport

Cyanide must never be stored or transported in the same transport unit together with acids or acid salts, oxidising agents, foods, foodstuffs, or anything else intended for human or animal consumption.

Cyanide solutions must only be transported on defined routes. The route to be followed is selected carefully and known to both the haulier and the consignor or supplier. As far as possible, the route should utilise motorways/highways and avoid major population centres.

3.3.1 Hydrogen cyanide

HCN is usually classified by governmental authorities as very poisonous, requiring special packaging and transport regulations. Similar strict procedures exist for solutions with an HCN content of 5% or more. Smaller quantities of the stabilised acid are transported in metal cylinders of up to 56 kg nominal water capacity in the USA and up to 60 kg in Germany. Cylinders cannot be charged with more than 0.55 to 0.60 kg of liquid HCN per litre of bottle capacity, and resistance to deformation must be tested up to 10 MPa before first filling. The water content should not exceed 3%, and storage time should be less than one year. According to national regulations, transport of quantities > 100 kg requires special permission. Procedures covering details of tank car size (up to 50 t), shipping, loading, and handling must be obeyed.

HCN is mainly shipped by rail under the provisions of the applicable transport regulations. Transport in railcars is permitted only with the approval of the competent authorities. The transport classifications of the most common transport regulations for HCN are: International Carriage of Dangerous Goods by Rail (RID) and European Agreement concerning the International Carriage of Dangerous Goods by Road (ADR) Class 6.1, 1°a, US (domestic transport) Code of Federal Regulations (CFR) 49 Class 6.1 and UN 1051 Class 6.1, packing group (PG) I ‘Poison inhalation hazard, hazard zone A’. Air transport is prohibited in accordance with International Air Transport Association (IATA) and International Civil Aviation Organization (ICAO) regulations (AtoFina, 2000a; Degussa, 1999; Gail *et al*, 2000; Ineos Acrylics, 2000b).

3.3.2 Sodium and potassium cyanide

Alkali metal cyanides can be shipped by road, rail, or sea freight under the provisions of the applicable transport regulations. The transport classifications of the most common transport regulations for alkali metal cyanide solid are: RID/ADR and European inland waterways

(ADNR [a]) Class 6.1, 41°a; International Maritime Dangerous Goods (IMDG), IATA/ICAO and CFR 49 Class 6.1 PG I; UN 1689 for NaCN and UN 1680 for KCN, both PG I (Degussa, 2000a,b; Gail *et al*, 2000; Ineos Acrylics, 2000c).

For alkali metal cyanide solutions, the regulations are: RID/ADR and ADNR Class 6.1, 41°a, b or c (depending on the concentration), IDMG Code and CFR 49 Class 6.1, and previous UN 1935, PG I, II, or III (depending on the concentration) (DSM, 1996; Dow Europe, 2001; Gail *et al*, 2000). Current UN numbers are 3414 for NaCN solutions and 3413 for KCN solutions (CEFIC, 2006).

Cyanide solutions should only be transported on defined routes. The route to be followed must be selected carefully and should be known to both the haulier and the consignor or supplier. As far as possible, the route should utilise motorways/highways and avoid major population centres.

3.3.3 Acetone cyanohydrin

ACH is often conveyed by closed pipeline between production and use facilities (most of the ACH produced is consumed on site for the manufacture of methacrylates).

ACH can be shipped by road, rail, or sea freight under the provisions of the applicable transport regulations. The transport classifications are: RID/ADR and ADNR Class 6.1, IDMG Code and CFR 49 Class 6.1, and UN 1541, PG I (AtoFina, 2000b; Ineos Acrylics, 2000; Röhm, 2000).

ACH should not be stored or transported in the same transport unit together with alkalis, alkali salts, foods, feedstuffs, or anything else intended for human or animal consumption.

Transport aboard a passenger-carrying aircraft is forbidden; the limit for single containers aboard a cargo aircraft is 280 litres (55 gallons) (Sifniades, 1987).

[a] Autorisation de transport de matières dangereuses pour la navigation sur le Rhin

3.4 Use

3.4.1 Hydrogen cyanide

HCN is used as an intermediate in the production of other substances. Major end uses include the production of adiponitrile, methyl methacrylate, cyanuric chloride, chelating agents, NaCN, and methionine and its hydroxy analogues.

3.4.2 Sodium and potassium cyanide

In gold extraction by cyanidation, the counter-cation has no effect on the leaching process. Therefore, the cheaper cyanides are used for this application. As KCN is currently more expensive than NaCN, the latter is used exclusively in this industry. In other applications, the counter-cation is of importance, and KCN has some advantages, e.g. in electroplating, because the potassium ion has greater mobility than the sodium ion (Gail *et al*, 2000).

The following text follows the review of Gail *et al* (2000), where references can be found.

Gold and silver leaching

The extraction of precious metals, such as gold and silver from ores, is the main application of NaCN. As a result of its unique chemical affinity, especially for gold, cyanide forms the most stable complex of all available lixiviants (chemical leaching agents), which is important for the complete recovery of the accessible gold in the ore. The high affinity for gold enables operation at relatively low cyanide concentrations while achieving a high recovery. Cyanidation has been known and applied for more than 100 years in all five continents, and the potential impact on the environment has been studied in detail. Suitable technology for effective cyanide detoxification is available and used. Efforts to replace cyanide with other, less toxic, complexing agents have remained unsuccessful. All other lixiviants, such as chloride/chlorine, bromide/bromine, hydrogen sulphide and thiourea, require much higher concentrations, more complicated processes and higher costs compared to the cyanidation technology. Furthermore, these reagents are also toxic, and their impact on the environment as well as suitable detoxification techniques have not yet been studied in any detail.

Base metal flotation

Cyanide is used as a depressant in the selective flotation of zinc, lead, copper and iron. This application is declining because other, higher performance and more economical compounds have gained in importance over the last few years.

Electroplating

Large quantities of alkali metal cyanides, as well as the complex cyanides of copper, zinc and cadmium manufactured from these alkali cyanides, are used in electroplating processes. In particular, the plating of iron, steel and zinc is carried out with cyanide-containing electrolytes. The main advantages are good corrosion-protection resulting from the even deposition of layers, ease of use, and low sensitivity of the bath to impurities. In this application, KCN is preferred, particularly for the deposition of gold and silver. Generally, electrolytes based on potassium salts have the advantages of better performance and lower sensitivity to impurities. When the highest quality is required, cyanide pretreatment and degreasing baths are still in use today. Attempts to employ other strong complexing agents have been hampered by wastewater problems.

Metal-surface hardening in molten salts

NaCN and KCN are important because they release nitrogen or carbon or both onto the surface of the material being treated at relatively low temperatures. The Tufftride process is employed primarily for the hardening of steel parts used as tools or in engines where they are highly stressed. Predominantly, NaCN is used in this application, because of its lower price.

Organic synthesis

Large quantities of NaCN are used to introduce cyano groups into organic compounds. This is the first step in the synthesis of a variety of well-known compounds used in pharmacy, complex chemistry and polymer chemistry. In particular, organic halogen compounds react with NaCN in water or polar aprotic solvents to form nitriles:

$$NaCN + RCl \rightarrow RCN + NaCl \quad \text{(Eq. 10)}$$

Where R = group like alkyl, aryl or alkoxy that that does not contain O–H or N–H

The nitriles can be converted to carboxylic acids, amides, esters, and amines. The sodium salt of cyano-acetic acid, for example, is manufactured in this way and is a raw material for the manufacture of malonic acid, caffeine and numerous other important organic products.

Inorganic chemistry

An important application of NaCN and KCN is the production of heavy metal cyanides, particularly complex iron cyanides.

3.4.3 Acetone cyanohydrin

By far the largest use of ACH is as an intermediate in the synthesis of methyl methacrylate, methacrylic acid and higher methacrylate esters. A small amount is converted to methacrylamide. The estimated amount of acetone used in the manufacture of methacrylate esters in 1980 in the USA was 280 kt. Based on this estimate, the quantity of ACH produced as an intermediate was approximately 400 kt (Sifniades, 1987). More recent figures on ACH consumption were not readily available. Data for global ACH demand can be purchased from PCI [a].

Minor quantities of ACH are used indirectly to introduce HCN into reactions (relatively safely due to its low volatility) and also directly as a hydrocyanidation agent. These reactions are non-specific and exploited in the manufacture of certain pharmaceuticals, pesticides and plant growth regulators (Sifniades, 1987; Röhm, 2000). This minor use represents an almost negligible fraction of the ACH market, compared to the total volume of ACH used in methyl methacrylate synthesis.

As HCN is highly volatile and highly flammable, it is often converted to the less volatile ACH for transport purposes.

[a] Annual "World hydrogen cyanide and derivatives, supply/demand report." PCI - Fibres & Raw Materials, Worth Corner, Turners Hill Road, Crawley, West Sussex RH10 7SL, England, UK (E-Mail: fibres@thepcigroup.com)

4. ENVIRONMENTAL DISTRIBUTION AND TRANSFORMATION

4.1 *Sources of cyanides*

Existing reviews for HCN typically mention human industrial activities as the principal anthropogenic sources of cyanide released into the environment. However, during this review, biomass (vegetation) burning was identified as the main contributor to the environmental release of HCN on a global scale. This section presents the available information on biomass burning, industrial, biogenic and other sources according to their relative magnitude, as far as known. An overview (quantification) of the sources and sinks of HCN is given at the end of this chapter (Section 4.4.2).

4.1.1 Biomass burning

Biomass (vegetation) burning as a source of HCN was first reported by Crutzen and Andreae (1990) and by Crutzen and Carmichael (1993). Biomass burning occurs during, or as a consequence of, shifting agriculture, permanent deforestation, savannah fires and disposal of agricultural waste. The amount of HCN released by biomass burning has been estimated to be between 0.2 and 6.13 Mt/y (Section 4.4.2).

Lobert and Warnatz (1993) have studied the emissions of biomass fires in relation to fire conditions. A distinction was made between the flaming and smouldering combustion stages. Low molecular mass nitriles, primarily HCN and acetonitrile, represent about 4% (average) of the nitrogen balance of biomass fires. Under flaming conditions, this is about 2.7% of the nitrogen content of the biomass, and under smouldering conditions 5.3%.

The release of high quantities of HCN into the environment through burning of biomass, such as wood, grass and leaves, was reported in many other studies (Yokelson *et al*, 1997), notably in south east Asia (Li *et al*, 2000; Tang *et al*, 2003) (Section 4.3.1). Ammonia is normally the major nitrogen-containing emission detected from smouldering combustion of biomass, but in emissions from some smouldering organic soils, experimentally fired, HCN was dominant (Yokelson *et al*, 1997).

Recent knowledge on the sources of atmospheric cyanide has been gained under the US National Aeronautics and Space Administration's (NASA) global tropospheric experiment (GTE), a commitment by the US government to understand the human impact on the global atmosphere. The GTE-experiments consisted of long-run flights with planes, extensively equipped for sampling and analysing traces of air contaminants at different heights of the air column. Several projects were part of the GTE, like the chemical instrumentation test and evaluation experiments

(CITE) and field studies as atmospheric boundary layer experiment (ABLE), transport and chemistry near the equator in the Atlantic (TRACE-A) and in the Pacific (TRACE-P) and the Pacific exploratory missions (PEM) west-A and west-B and PEM-tropics. Figure 3 and Table 5 may give an impression of the efforts involved in atmospheric monitoring above the continents of Africa, America, Asia and above the Pacific Ocean (NASA, 2005).

Figure 3: US-NASA global tropospheric experiment (GTE) (NASA, 2005)

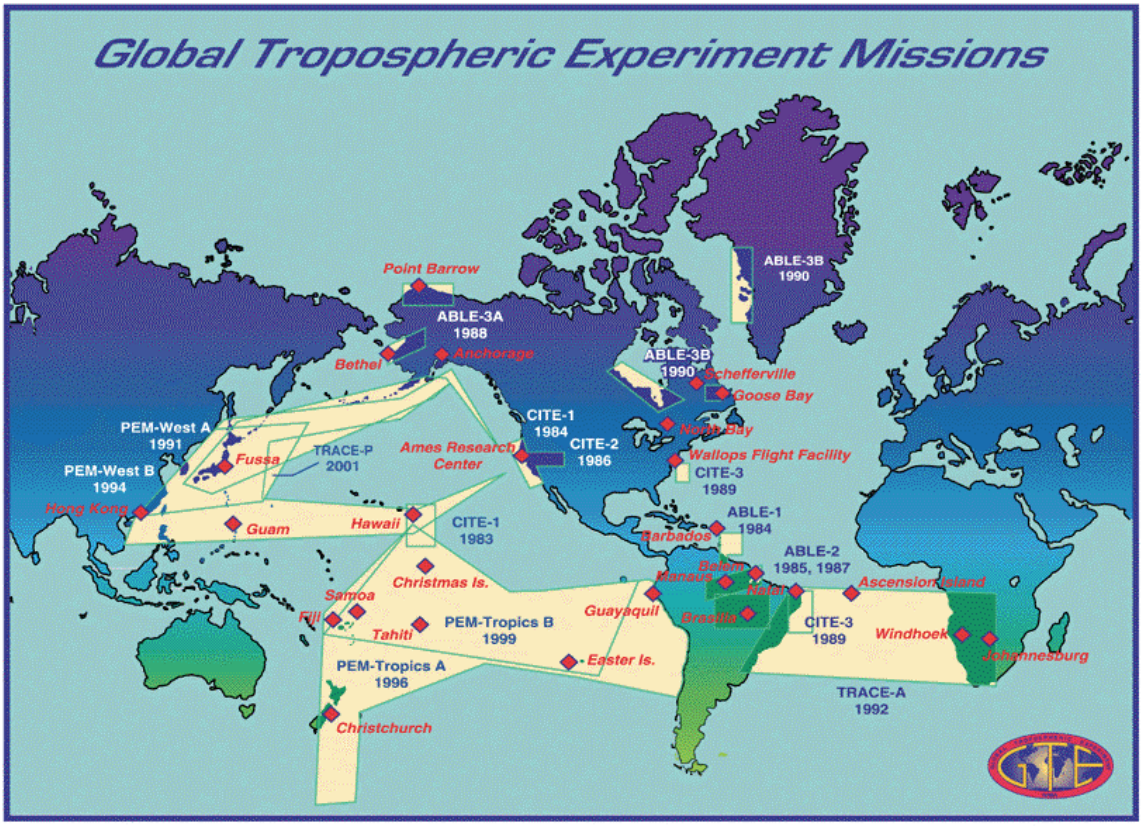


Table 5: US-NASA Global Tropospheric Experiment (GTE) (NASA, 2005)

Mission [a], location	Objective	Aircraft	Date of completion
ABLE-1 Barbados	Boundary layer chemistry and dynamics-precursor	Electra	June 1984
ABLE-2A Brazil	Boundary layer study of $CO/O_3/NO_x$ - dry season	Electra	Aug. 1985
ABLE-2B Brazil	Boundary layer study of $CO/O_3/NO_x$ - wet season	Electra	May 1987
ABLE-3A Alaska	Photochemistry and biogenic sources of tropospheric gases	Electra	Aug. 1988
ABLE-3B Canada	Photochemistry and biogenic sources of tropospheric gases	Electra	1990
CITE-1A Wallops Island, Virginia	Ground-based instrument inter-comparisons	Not applicable	July 1983
CITE-1B Tropics	Airborne instrumentation inter-comparisons	CV-990	Nov. 1983
CITE-1C Tropopause fold	Airborne instrumentation inter-comparisons	CV-990	Apr. 1984
CITE-2 West coast USA	Test and inter-comparisons: nitrogen budget experiments	Electra	Aug. 1986
CITE-3 Tropical Atlantic	Inter-comparisons of sulphur budget experiments	Electra	Sept. 1989
PEM-Tropics A South central tropical Pacific	Ozone and sulphur photochemistry/transport of gases and aerosols	DC-8 and P-3B	Aug./Sept. 1996
PEM-Tropics B Central and eastern tropical Pacific	Ozone and sulphur photochemistry/transport of gases and aerosols	DC-8 and P-3B	Mar./Apr. 1999
PEM-West-A Western Pacific	Photochemistry and transport of gases and aerosols	DC-8	1991
PEM-West-B Western Pacific	Photochemistry and transport of gases and aerosols	DC-8	Feb./Mar.1994
TRACE-A Tropical Atlantic	Transport and photochemistry of ozone in the tropics	DC-8	1992
TRACE-P Western Pacific	Transport and photochemistry of gases and aerosols	DC-8 and P-3B	Feb.-May 2001

[a] Full names in text

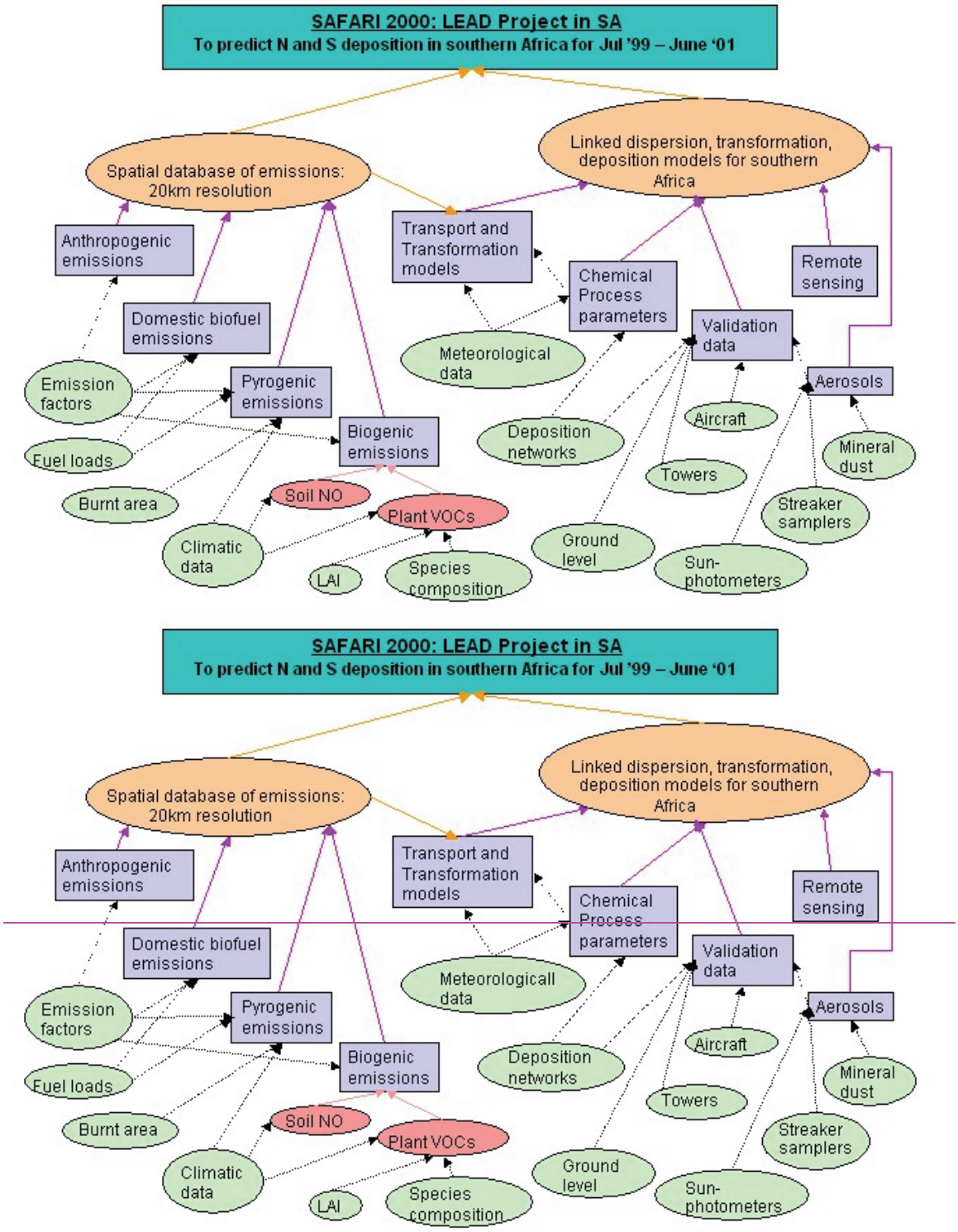
In the USA, most of the fires are due to prescribed burning in the southeast and most of the fires in the extra-tropical northern hemisphere occur in the boreal forest. In fact, boreal forest fires exert a major influence on the summertime atmospheric chemistry of much of the northern hemisphere. During 1997, in North Carolina and Alaska, HCN was among 15 of the most common components measured in smoke plumes from single, large fires. Concentrations in two samples of a plume from a slow-moving surface fire (probably started by lightning) were 0.024 and 0.047 ppm (Goode *et al*, 2000).

A linear relationship was found between the concentrations of HCN (data in Section 5.1.1) and methyl chloride (CH_3Cl), a known specific tracer of biomass combustion, and CO and potassium as generic tracers of biomass burning, in 12 fresh (< 5 days old) plumes sampled *in situ* (TRACE-P) in the free troposphere (> 3 km) over the western and central/eastern Pacific regions. This supported the view that the atmospheric burden of HCN is strongly associated with biomass burning sources. The relationship was maintained over the entire Pacific troposphere and persisted in the higher troposphere (8 - 12 km), but broke down in the boundary layer due to the complexity of Asian anthropogenic sources and proximity of an ocean sink (Section 4.3.1) (Singh *et al*, 2003).

Using the enhanced ratio of HCN (and acetonitrile [CH_3CN]) concentration relative to CH_3Cl or CO (a generic tracer of smoke), 5-day back trajectories indicated the origin of the 12 free (> 3 km) plumes over regions of southern China, southeast Asia and northern Africa (satellite observations also showed fires in these regions at the time). All tracers of biomass combustion (HCN, CH_3CN, CO, CH_3Cl and potassium) were significantly elevated in these plumes, while the contribution of urban pollution was small (perchloroethylene [C_2Cl_4] < 10 ppt) (Singh *et al*, 2003). The global sources are quantified in Section 4.4.2.

A similar project to TRACE-P was the southern African regional science initiative (SAFARI) 2000 project. This was an international science initiative aimed at developing a better understanding of the southern African earth-atmosphere-human system. The goal of SAFARI 2000 was to identify and understand the relationships between the physical, chemical, biological and anthropogenic processes that underlie the bio-geophysical and biogeochemical systems of southern Africa. Particular emphasis was placed upon biogenic, pyrogenic and anthropogenic emissions, their characterisation and quantification, their transport and transformations in the atmosphere, their influence on regional climate and meteorology, their eventual deposition, and the effects of this deposition on ecosystems. The complexity of the SAFARI 2000 project is illustrated in Figure 4 (Swap *et al*, 2002; ORNL, 2005).

Figure 4: Overview of the SAFARI 2000 project



The figures below show the study area of SAFARI 2000 in southern Africa and a typical flight track for sampling air masses in the smoke plume released from a fire (Figures 5 and 6).

Figure 5: SAFARI study area
(Swapp *et al*, 2002)

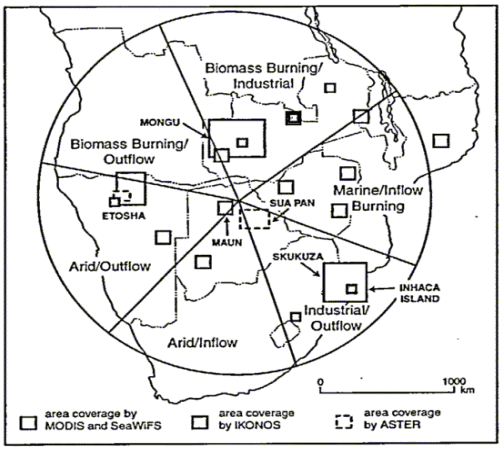
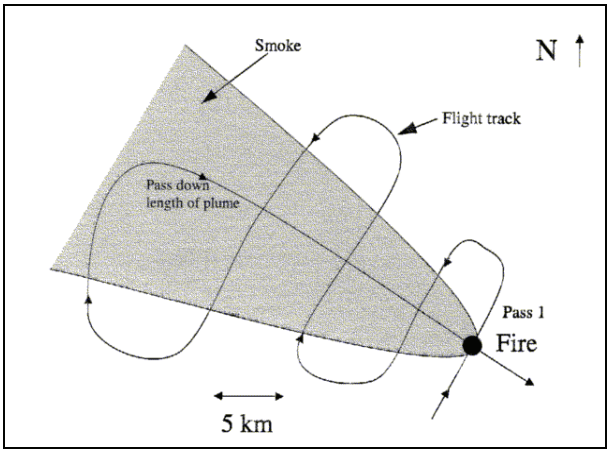


Figure 6: Track for plume sampling in SAFARI
(Hobbs *et al*, 2003)



During the dry season campaign of SAFARI 2000, prescribed biomass burning was conducted in several southern African countries, representing the main vegetation types. Measurements were made on the ground, with aircraft and by satellite. The fires ranged from ten to several thousand hectares in extent. Fuel loads and fuel moisture contents were sampled before ignition, while weather conditions and fire behaviour components such as height of flame and length of fire front were recorded during the burns in order to assist in validating combustion products by spectrophotometric analysis by the polar orbiting satellite, Terra. The ratio of HCN to CO_2, released from the biomass fire, was found to be about 5×10^{-4} on average. Actual levels up to 37 ppb of HCN were observed (see Table 3 in Hobbs *et al*, 2003).

Barber *et al* (2003) investigated cyanide releases from biomass burning through laboratory tests and field sampling. Duplicate laboratory combustion tests were conducted under normal open burning conditions and under conditions of reduced airflow. Field sampling was conducted following the Waterville Complex wildfire in the USA, which burned 538 hectares in Tennessee and North Carolina near the Smoky Mountains National Park in November 2000.

During experimental laboratory combustion of biomass, 25 to 31 mg of HCN was released per kg dry matter. The ash was leached with simulated rainwater with a liquid-solid ratio of 20:1. The available (total) cyanide level in the leachate of residual ash varied from 40 to 135 µg/l; free cyanide was not detected. The total burden of cyanide in the ash was < 1% of the cyanide

released during the simulated fire. However, free cyanide concentrations in the run-off of burned areas amounted to as much as 49 µg/l. Although gaseous HCN was expected to disperse rapidly following aerial emission, rainout of the emitted HCN contributed significantly to concentrations in run-off. The release of volatile cyanides observed by Barber *et al* (2003) was lower than expected from the HCN/CO_2 ratio observed in the plume of fire gases (Goode *et al*, 2000; Hobbs *et al*, 2003).

Conclusion on release of HCN from nitrogen in biomass

The TRACE-P and SAFARI 2000 projects have revealed that considerable amounts of HCN are released from biomass (vegetation) burning. To obtain an idea of the extent of conversion into HCN without knowing the exact nitrogen content of the vegetation, it is assumed that 1% (by weight) of nitrogen (N) and 60% carbon (C) by weight of the vegetation is combustible. This is equivalent to a molar C/N-ratio of 70. If all nitrogen is combusted to HCN and all carbon to CO_2, one would expect a ratio of HCN/CO_2 of 1.4×10^{-2} by volume. In practice, a lower ratio of 0.05×10^{-2} is frequently observed, i.e. 3.5% of nitrogen from biomass burning is converted into HCN. This is in agreement with Lobert and Warnatz (1993), who estimated 2.4% conversion into HCN and 1.6% into acetonitrile.

In the TRACE-P project, it was concluded that the atmospheric HCN above the Pacific Ocean originated from biomass burning. There exists a linear relation between methyl chloride (a tracer of biomass combustion) and HCN, acetonitrile and CO in the free troposphere over the western, central and eastern Pacific regions. On the basis of a decreasing concentration of HCN and acetonitrile from large to small height above the sea surface, it was inferred that oceanic loss is a dominant sink for these cyanides. The annual deposition loss to the oceans was estimated to be 1.4 Tg (teragrammes) of nitrogen (1.4 Mt N) from HCN (72%) and acetonitrile (28%). This is equivalent to a deposition of 1.95 Mt of HCN and 1.14 Mt acetonitrile, which is released annually from biomass burning (Singh *et al*, 2003).

Tobacco smoking

Following IARC (1986 p. 96), and references cited therein, "HCN is one of the most toxic agents in the vapour phase of tobacco smoke. Model studies indicate that nitrate is an important precursor for HCN in smoke and that tobacco proteins, especially glycine, proline and aminodicarboxylic acids, also give rise to HCN (Johnson and Kang, 1971; Johnson *et al*, 1973a). Tobacco smoke also contains µg amounts of cyanogen $(CN)_2$, which hydrolyses during analytical procedures and thus contributes to the total amount of HCN found (Brunnemann *et al*, 1977a).

The mainstream[a] [smoke] of commercial cigarettes contains 160 to 550 µg HCN/cigarette; however, the mainstream emission of HCN in the smoke of cigarettes with filter tips containing charcoal, with perforated filter tips or with filter tips with longitudinal air channels is low (< 100 µg) (Johnson *et al*, 1973b; Brunnemann *et al*, 1977a; Sloan, 1980; Jenkins *et al*, 1983; Norman *et al*, 1983). The release of HCN into sidestream[b] [smoke] is significantly lower than that into mainstream [smoke] (14 - 134 µg/cigarette) (Johnson *et al*, 1973b; Brunnemann *et al*, 1977a). The concentration of thiocyanate in the saliva, blood and urine of smokers is often used as an indicator for the uptake or depth of inhalation of tobacco smoke (Benowitz, 1983)."

The level of HCN found in cigarette smoke under ISO standard smoking conditions was 32 to 156 µg/cigarette in mainstream smoke and 77 to 136 µg/cigarette in sidestream smoke (Health Canada, 1999, 2003).

The amount of HCN in mainstream (inhaled) smoke has been calculated to range from 10 to 400 µg per cigarette, with the ratio of cyanide concentration in sidestream smoke to mainstream smoke ranging from 0.006 to 0.27. Thus, exposure of smokers could range from 10 to 40,000 µg HCN/d (Surgeon General, 1979 cited by Fiksel *et al*, 1981 p. 6-25). HCN concentrations measured in mainstream and sidestream smoke ranged from 280 to 550 µg/cigarette and 53 to 111 µg/cigarette, respectively (Norman *et al*, 1983 cited by Guerin *et al*, 1987). Sidestream-mainstream ratios of HCN concentrations ranged from 0.6 to 0.37 (Surgeon General, 1982 cited by Fiksel *et al*, 1981), 0.17 to 0.37 (Surgeon General, 1982 cited by Guerin *et al*, 1987) or 0.06 to 0.50 (Baker and Proctor, 1990). The latter information may be used to calculate passive smoking exposure (room concentrations and body burden/uptake).

In 1976 in the USA, the estimated amount of cyanide released in the air from cigarette smoke was 13,000 to 750,000 pounds[c] (5.9 - 340 t HCN) (Fiksel *et al*, 1981 cited by ATSDR, 1998).

HCN is released from a burning cigarette into the environment via the sidestream smoke. Guerin *et al* (1987) compiled data from several sources and concluded that the release of HCN in sidestream smoke seldom varied more than a factor 2 to 3, depending on the type of cigarette. A good estimate of sidestream HCN was 100 µg/cigarette. Thus, smoking of the total world production of 5.61×10^{12} cigarettes in 1998 (ASH, 2004) might have released 560 t HCN into the environment. Based on 10 to 400 µg HCN/cigarette (Surgeon General, 1979 cited by Fiksel *et al*, 1981 above), global emissions would range from 56 to 2,240 t HCN. Both estimates are a fraction of the HCN released from global biomass burning (Section 4.4.2).

[a] Mainstream smoke from cigarettes and cigars is generated during puff-drawing in the burning cone and hot zones; it travels through the tobacco column and exits from the mouthpiece

[b] Sidestream smoke is formed in between puff-drawing and is emitted freely from the smouldering tobacco product into the ambient air

[c] 1 lb = 0.4535924 kg

US cigarette consumption in 2001 was 398.3×10^9 cigarettes (US-FTC, 2005). In China in 1999, 320×10^6 smokers (1/3 of the world's smokers) smoked 2.2×10^{12} cigarettes. World-wide, the World Health Organization (WHO) estimates the number of cigarettes smoked per year to 5.61×10^{12} cigarettes (1.1×10^9 smokers) (ASH, 2004).

4.1.2 Industrial sources

This section reviews the available information on industrial sources of HCN, including production, use and processing, waste disposal and accidental releases during production and use. The available emission inventories are discussed.

HCN concentrations in arctic air over Spitsbergen have been measured since 1992. A peak observed in 1998 could be traced back to air masses advected over Siberia, northern America, the major industrialised areas of Russia, and Western Europe (Velazco *et al*, 2003).

The European pollutant emission register (EPER) reported the amount of HCN released to air in the EU in 2001 as approximately 157 t; emission of total cyanide to water was 343 t (≈ 260 t/y direct and ≈ 83 t indirect discharges) (EPER, 2004).

The US toxics release inventory (TRI) reported a total release of 2,830,000 pounds (1,284 t) of HCN in 2001. This broke down, using their categorisation by sector, to an estimated total of 1,190,000 pounds (539 t) of HCN emitted to air, 235 pounds (0.107 t) to surface water, 934 pounds (0.424 t) to land and 1,700,000 pounds (771 t) to 'on-site' underground injection (US-EPA, 2004a). The TRI data should be viewed with caution since only certain types of facilities were required to report, rendering the list incomplete. Moreover, no information was available in the TRI database for other cyanides and thiocyanates, compounds excluded from reporting under Title III of the Superfund Amendments and Reauthorization Act (SARA) (US-EPA, 1993 cited by ATSDR, 1998).

Primary production

In the USA, the second largest source of cyanide emission to the air, after car exhaust, was the manufacture of methyl methacrylate, acrylonitrile and HCN (Fiksel *et al*, 1981 cited by ATSDR, 1998).

The TRI report on amounts of HCN released in 2001 by US industry facilities indicates that, contrary to the picture reported by Fiksel in the early 1980s, the majority of HCN from manufacture of HCN (87%) and acrylonitrile (91.3%) was injected 'on-site' into the ground

rather than being discharged to air. Further analysis of the data (interrogation of the individual facilities) showed that the greatest part of the combined total of 10,955 pounds (5.0 t) reportedly released by methyl methacrylate and ACH production facilities (including some acrylonitrile production) was from ACH rather than methyl methacrylate production. Overall, in the USA in 2001, primary production of HCN, ACH and acrylonitrile accounted for 11.9%, 9.4% and 7.0%, respectively, of total HCN releases by the industry to air, compared with 35.9% from carbon black production and 27.4% from gold mining (Table 6) (US-EPA, 2004a).

No data are available for HCN released from primary production of HCN, ACH and acrylonitrile in Europe.

HCN emission during production of NaCN and KCN at a typical European plant (20 kt/y, > 5,000 working h/y) is approximately 7.5 kg of HCN/y. This is far below the German HCN maximum limits of 50 g/h and 5 mg/m^3 (TA-Luft, 1986 p. 47). NaCN and KCN dust formed during the production process is absorbed in a solution containing excess caustic soda.

Table 6: Releases of HCN by the chemical industry in the USA in 2001, reported by the US toxics release inventory (Adapted from US-EPA, 2004a)

Industry	Air emission			Surface water discharge			Underground injection		
	(lb)	(t) [a]	(%)	(lb)	(t) [a]	(%)	(lb)	(t) [a]	(%)
Carbon black production	404,053	183	35.9	0	0	0	0	0	0
Gold mining	308,907	140	27.4	0	0	0	0	0	0
HCN production	133,615	61	11.9	0	0	0	868,797	394	51.1
ACH/methyl methacrylate production	105,955	48	9.4	0	0	0	0	0	0
Acrylonitrile production	79,285	36	7.0	0	0	0	828,000	376	48.7
Multiple industries	37,759	17	3.4	0	0	0	0	0	0
Organic fibres	31,157	14	2.8	0	0	0	0	0	0
Others	26,172	12	2.3	235	0.11	100	0	0	0
Total	1,126,903	511	100	235	0.11	100	1,698,997	771	100
%	40			0.0008			60		
Grand total	2,826,135	1,282							

[a] Converted (1 lb = 0.4535924 kg) and rounded values

Use and processing

Cyanide emissions from its use can be related to the mining, metal plating and metal finishing industries.

It is estimated that 330 kt NaCN/y are used by mining operations globally (Section 3.1.1). HCN emissions from these operations to air cannot be estimated due to the absence of a standard emission factor, but are dependent on local operating conditions (type of ores, photolysis in tailings ponds, effluent treatment).

Korte and Coulston (1998) published a figure of 22 kt/y of HCN released from gold mining operations due to the use of NaCN. This would represent 10% of NaCN used by the mining industry.

Secondary production

HCN is formed during dry distillation of nitrogen containing materials. In particular, coal in coking may give rise to HCN during normal operation of steel blast furnaces. It is often also present in generator gases. Raw cokery off gas contains about 1% HCN, or cyanogen $(CN)_2$ that will form HCN in living organisms (Seeger and Neumann, 1988). Other coal carbonisation processes also release HCN into the atmosphere (Cicerone and Zellner, 1983).

ATSDR (1998), and references therein, reported other smaller sources of cyanide release from thermic processes like iron and steel production, coal combustion (Fiksel *et al*, 1981), petroleum refining (Fiksel *et al*, 1981), oil shale retorting (Hargis *et al*, 1986; Sklarew and Hayes, 1984) and municipal solid waste incineration (Carotti and Kaiser, 1972; Greim, 1990). In 1976 in the USA, 18,000 to 180,000 pounds (8.2 - 81.6 t) were released from waste incineration and an estimated 137,000 pounds (62 t) from certain agricultural pest control activities (Fiksel *et al*, 1981).

The US-TRI reported releases to air of 404,053 pounds (183 t) of HCN from carbon black production and 308,907 pounds (140 t) from gold mining in 2001. Other industries accounted for less than 8.5% of the total industrial discharges to air, including electrical equipment (35,759 pounds/16 t), petroleum refining (6,501 pounds/2.9 t), transport equipment (4,136 pounds/1.9 t) and primary metals (2,828 pounds/1.3 t) (US-EPA, 2004a). The total of these airborne emissions is 345 kt.

The European pollutant emission register (EPER) reported that the main direct releases of total cyanides to water within the EU in 2001 were from the metal industry and metal ore roasting or sintering installations together with installations for the production of ferrous and non-ferrous

metals (204 t). The next most significant sources were associated with basic organic chemicals (24 t) and basic inorganic chemicals or fertilisers (21 t). Other sources were mineral oil and gas refineries (4,518 kg), plants for the production of milk or animal raw materials or vegetable raw materials (2,550 kg), industrial plants for pulp from timber or other fibrous materials and paper or board production (1,414 kg), installations for the disposal or recovery of hazardous waste or municipal waste (520 kg), pharmaceutical products (420 kg), coke ovens (224 kg), installations for the production of cement clinker, lime, glass, mineral substances or ceramic products (204 kg), installations for the disposal of non-hazardous waste and landfills (71 kg), biocides and explosives (64 kg). The analysis was based on 12 different activities and 102 facilities within the EU. The country with the highest releases was Spain (153 t) (EPER, 2004).

EPER also reported 2001 emissions of HCN to air from: basic organic chemicals (60 t), coke ovens (50 t), and metal industry and metal ore roasting or sintering installations together with installations for the production of ferrous and non-ferrous metals (40 t). Other activities included mineral oil and gas refineries (1,220 kg), plants for pretreatment of fibres or textiles (541 kg) and pharmaceutical products (427 kg). The analysis was based on 7 activities at 33 facilities within the EU. The country with the highest releases was the UK (104 t) (EPER, 2004). The total of these airborne emissions is 102 kt.

It should be noted that EPER cautions that 2001 was the first reporting year for the register and the data are not complete for all activities and pollutants.

Waste disposal

Volatilisation of cyanide from waste disposed of in landfills has been reported (Fiksel *et al*, 1981).

Cyanides (reported as cyanide, HCN, NaCN, KCN, calcium cyanide, or copper[I]- cyanide) have been detected in air samples collected at 5 of the 406 hazardous waste sites in the USA where cyanides have been detected in at least one environmental medium. The HazDat information used includes data from both National Priorities List and other Superfund sites (HazDat, 1996 cited by ATSDR, 1998).

EPER reported that, within the EU in 2001, 520 kg of total cyanides was disposed of to water at installations for the disposal or recovery of hazardous waste or municipal waste and 71 kg at installations for the disposal of non-hazardous waste and landfills (EPER, 2004).

Accidental releases during production and use

There have been some reports of incidents and accidents involving cyanide in Europe. These can be divided into two broad categories depending upon whether they occur during transport or during end-use.

The German 'Gefahrgutunfall-Datenbank im Internet' (GUNDI) hazardous chemicals database reported four transport incidents involving cyanide in Europe since 1992 (of a total of 1,717 incidents involving chemicals). Two of these involved small amounts of product loss and these were without any major consequence (GUNDI, 2004). This database, although only citing articles from German newspapers, may offer representative statistics for a European country. Another example of a transport incident in Europe in recent years was the derailment of a train that was transporting a rail wagon of aqueous NaCN in Belgium close to the Dutch border in 2000. No product loss or spillage occurred in this case. Although the available information is likely to be incomplete, it suggests that there have been very few transport accidents or incidents involving cyanides in the last decade.

The second broad category of incidents and accidents that can occur, with potentially greater consequence, is the loss of containment at end-user sites. The largest incident involving release of cyanide in Europe in recent years was the Baia Mare incident in Romania in 2000. In this incident approximately 100,000 m^3 of wastewater from a broken tailings dam containing approximately 100 t of cyanide (most of it complexed to copper) entered the river system feeding the river Danube and caused the death of many aquatic life-forms in the whole river system (UNEP-OCHA, 2000).

Although it was apparently the only major incident recorded over the last two decades, the scale of the Baia Mare incident triggered the development of the international Cyanide Management Code (2003) under the guidance of the UN Environmental Program (UNEP; draft guidelines and audit protocols of April 2003) and an extension of the Seveso II Directive (modification of Directive 96/82/EC) and a Mining Waste Directive to include tailings pond facilities (CEC, 2003, 2006).

Emission inventories

There are several inventories worldwide that collect data on industrial emissions of dangerous substances to land, air and water. These inventories contain prescribed lists of chemicals, which have to be reported to the national environmental agencies if specific emissions limits are exceeded. These limits are based on mass rather than concentration. HCN and cyanides are listed in all of the registers mentioned here. Any HCN emission from specified industries and process

applications is covered and has to be reported. The registers also address the HCN emissions from mining facilities using cyanides for gold and silver leaching.

The data are compiled regularly for specific reporting periods, and published mainly on the internet. Examples are:

- Australia: national pollutant inventory (NPI), www.npi.gov.au
- Canada: national pollutant release inventory (NPRI), www.ec.gc.ca/pdb/npri
- Europe: European pollutant emission register, www.eper.cec.eu.int (discussed above)
- UK: pollution inventory (formerly inventory of sources and releases, ISR), www.environment-agency.gov.uk/business/444255/446867/255244
- USA: toxics release inventory (TRI), www.epa.gov/tri (discussed above)

Some of the registers, such as TRI (USA) and NPI (Australia), also require ammonia (NH_3) emissions (originating from a process using cyanide) to be reported as HCN. This 'over-reporting' may not always reflect the real HCN emissions to air. It should be possible to better characterise and quantify the various emissions in future, when these registers have gathered sufficient data on HCN releases world-wide and in Europe over a longer period of time.

4.1.3 Biogenic sources

Nitriles (–C≡N) are widely distributed among living organisms, including many higher plants, algae, bacteria, fungi, invertebrates, and also mammals (leukocytes). These biogenic sources of HCN and CN^- have been reviewed here by way of examples and extracts from secondary literature sources (reviews).

Biological systems also have the capacity (enzymes or otherwise) to hydrolyse or degrade cyanide or nitriles (Section 4.3.4). The formation and degradation of cyanides often both occur in the same organism.

Plants

Many higher plants contain 'cyanogenic glucosides', i.e. glucosides of α-hydroxynitriles (Conn, 1979; Cooke and Coursey, 1981; Nartey, 1981; Seigler, 1981; all cited by Seeger and Neumann, 1988; Egekeze and Oehme, 1980). Early reports are of 2,000 plant species which are cyanogenic and 28 known cyanogenic glucosides, which are derived from 5 amino acid precursors (L-valine, L-isoleucine, L-leucine, L-phenylalanine and L-tyrosine [Conn, 1981]) by decarboxylation and conversion of the α-amino group to a nitrile (Knowles, 1976). More recent estimates mention

about 12 cyanogenic glucosides that have been found in over 800 different plant species. Most of these are not foods, but cyanogenic glucosides are found in some plants that are widely used as food (Bickerstaff, 2003). Well-known examples are the tubers of the tropical cassava (manioc) plant (Figure 7) and Lima (moon) beans. European examples are bitter almonds and kernels of apricot, cherry and peach fruits.

Figure 7: Cassava (IITA, 2005)

Most of these plants are able to produce HCN by acid hydrolysis or by enzymatic action of microbial glucosidases (US-EPA, 1980; Seeger and Neumann, 1988). For example:

$$C_{20}H_{27}NO_{11} + 2\ H_2O \xrightarrow{\text{Emulsin}} 2\ C_6H_{12}O_6 + C_6H_5CHO + HCN \quad \text{..... (Eq. 11)}$$

(Amygdalin + Water ⟶ Glucose + Benzaldehyde + Cyanide)

Amygdalin is the glucoside occurring in apricot pits (*Prunus armeniaca*) and many other members of the Rosacea family (amygdalin is also used as alternative anticancer agent). The chemical structure includes two glucose units and the nitrile group (–C≡N). Emulsin, the accompanying enzyme, can also hydrolyse several other cyanoglucosides, but not linamarin (in flax, below). Certain plants appear to contain the hydrolysing enzyme without corresponding glucoside. Pseudo-cyanogenic plants like cycads (e.g. *Cycas revoluta*) form glucosides with a $–N=N^+(OH)–CH_3$ group, rather than a nitrile group (Alston and Turner, 1963 and references therein).

The exact physiological role and significance of HCN production by plants and algae, and by other living organisms, is unclear and subject to discussion beyond the scope of this review. An

ecological role of cyanogenesis is recognised: it may offer the producer a selective advantage, e.g. as a chemical defence against herbivores and pathogens since HCN is a potent inhibitor of cytochrome *c* oxidase and several other metallo-enzymes (Blumer and Haas, 2000). For example, the Australian sugar gum (*Eucalyptus cladocalyx*) is cyanogenic due to the presence of prunasin in combination with a β-glucosidase-like enzyme. The concentration of prunasin was highest in young leaf tips, suggesting a role in the plant's defence against herbivores (Gleadow, 1999).

Releases of cyanide from plants to the environment may occur under specific culture conditions or during degradation of these materials when the plants are injured, in particular during the processing of cyanogenic food crops. HCN in natural waters partly stems from decomposing cyanogenic plant material (Krutz, 1981; Seeger and Neumann, 1988; Chew and Ip, 1990) (concentration levels are given in Section 5.1.2). To date, there are no data to enable a quantification of these sources. While releases of HCN from cyanogenic glucosides may be of concern for human health, there are no data to conclude that they are likely to be a major contributor to the environmental load.

HCN may be released upon wilting, frosting or stunting of plants and seeds, and the potential for high glucoside levels is greatest in fast-growing and immature plants (Egekeze and Oehme, 1980). The concentration depends on the tissue examined and varies greatly between varieties and also with environmental and cultural conditions (typical levels are reported in Section 5.1.4). The normal range of cyanogen content of cassava tubers falls between 15 and 400 mg HCN/kg fresh weight (Coursey, 1973 cited by FAO, 1990) and of Lima beans between 15 and 500 mg HCN/kg fresh weight (Bickerstaff, 2003). Bitterness is not necessarily a reliable indicator of cyanide content (FAO, 1990). HCN can be released from a cyanogenic glucoside in food by β-glucosidase either of plant or gut micro-flora origin (WHO, 1993).

Examples of cyanoglucoside containing plants that are not foods are: flax (*Linum usitatissimum*), sorghum (*Sorghum sudanese*, *S. bicolor*) and yew *Taxus baccata.* The levels of cyanoglucoside vary greatly depending on environmental (e.g. light) and genetic factors, and interaction between these factors. Even diurnal variation in HCN occurred in flax seedlings (Alston and Turner, 1963 and references therein).

Several species include discrete cyanogenic, acyanogenic, and intermediate cyanogenic varieties due to different genotypes, for example white clover (*Trifolium repens*) that can be related to one parent individual (dominant genotype) (Alston and Turner, 1963 and references therein; Hughes, 1981). In the perennial *Lotus corniculatus*, cyanide was particularly present in years with warm and dry weather conditions, but the variety 'major' was always free of cyanogen and the corresponding enzyme (Alston and Turner, 1963 and references therein). A population of *Eucalyptus cladocalyx* (Kangaroo Island, Australia) included cyanogenic plants only, but with widely varying concentrations of prunasin in leaf tissue. There was no clear association with

environmental factors such as rainfall, soil type or proximity to the coast, suggesting cyanogenic polymorphism (Gleadow 1999).

Some plants use cyanide excretion to regulate their root microflora.

Application of granular insecticides (carbofuran and phorate) around the roots of sorghum plants decreased the level of cyanoglucosides in roots and shoots. The lack of HCN caused a decrease in the fungal flora (*Aspergillus* and other species) and an increase in the bacterial *Azobacter chroococcum* population surrounding the roots (Kandasamy *et al*, 1977). The combined fungicidal and bacterio-incremental effects of HCN exudate in the rhizosphere of sorghum have been reported earlier; actinomycetes were not affected (Rangaswami and Balasubramanian, 1963).

A similar 'rhizosphere effect' was observed with a flax (*Linum usitatissimum*) variety resistant to *Fusarium lini* wilt. The roots of this variety were found to excrete or diffuse HCN, from hydrolysis of the glucoside linamarin, which lowered the incidence of fungi such as *Fusarium* and *Helminthosporium*. However, the growth of *Trichoderma* and other fungi was stimulated in the vicinity of the roots. The amounts of HCN excreted were given as 25.3 to 37.6 mg/plant. In contrast, the susceptible flax variety produced "only a trace" of HCN, and no shift in fungal flora (Timonin, 1941).

Preparation of cyanide-containing raw plant materials for food and industrial purposes

HCN is a volatile compound. It evaporates rapidly in the air at temperatures over 28°C and dissolves readily in water. It may easily be lost during transport, storage and analysis of food specimens. It can also be destroyed by boiling, roasting, expression, or fermentation. The glucoside can be removed (detoxified) by juice extraction, heating, fermentation, drying, or a combination of these processing treatments.

Traditional processing and cooking methods for cassava can, if efficiently carried out, reduce the cyanide content to non-toxic levels. Squeezing the product is a fundamental step in the elimination of the soluble cyanides. Soaking in water improves detoxification as cells are broken by osmosis and fermentation, which facilitates hydrolysis of the glucosides. Short soaking (four hours) is ineffective, but when longer periods are used (18 to 24 hours) cyanide levels can be reduced by 50%. The liberated HCN will dissolve in the water when fermentation is effected by prolonged soaking, and will evaporate when the fermented cassava is dried (FAO, 1990).

Cooke and Coursey (1981) reported a global production of cassava of 100 Mt. Based on the content of 0.4 to 0.5 g HCN/kg of cassava roots (Nartey, 1981) this could result in an

environmental release of HCN of up to 50 kt of HCN during processing. Other foods which contain cyanogenic glucosides (Lima beans, Sorghum, bamboo, *Vicia* beans) are also potential sources of environmental releases of HCN during processing, but estimates of production volumes are currently not available.

Green plants (and algae) are also capable of producing cyanide, in minute amounts, through a different mechanism, as follows.

Algae and grana

Micro-algae and grana [a] from green plant leaves do not contain cyanogenic glucosides, but they are able to form cyanides by a different mechanism involved in the coordination of nitrate assimilation and CO_2 fixation (photosynthesis). The cyanide needed for reversible inactivation (by HCN complexation) of nitrate reductase, may be formed from glyoxalate oxime, a substrate derived from glyoxalate and hydroxylamine, as demonstrated in extracts from the green alga *Chlorella vulgaris* and grana from spinach leaves *(Spinacea oleracea).* The cyanide forming enzyme(s) depends on ADP and requires cofactors such as manganese. Maximum cyanide formation (31.2 nmol HCN/300 ml/h = 0.1 µmol/l/h), by the isolated enzyme system, was obtained in the presence of adenosine triphosphate (ATP) (Solomonson and Spehar, 1981). Other cyanogenic precursors, in *C. vulgaris* extracts, were D-histidine (L-histidine less so), a substrate for D-amino acid oxidase with suitable cofactors such as peroxidase or cytochrome *c*, and malonodinitrile in grana from New Zealand spinach (*Tetragonia expansa*). HCN production was stimulated by high light intensity combined with high O_2 and low CO_2 pressures (Vennesland *et al*, 1981).

Other, non-physiological compounds can equally serve as substrate. Small additions of ferricyanide, for example, caused complete cessation of nitrate uptake and inactivation of cellular nitrate reductase in green cells (spinach grana and *C. vulgaris*). Again, HCN production was stimulated by illumination and O_2 (Vennesland *et al*, 1981).

Bacteria (including cyanobacteria)

Bacterial cyanogenesis occurs, as far as is known, only in few species, including *Chromobacterium violaceum*, fluorescent pseudomonads like *Pseudomonas aeruginosa* and *P. fluorescens*, free-living *Rhizobium leguminosarum*, and certain cyanobacteria (blue-green

[a] Granum, stack of thylakoid disks in chloroplast that contains chlorophyll

algae, below). According to Castric (1981), not all strains may have cyanide producing capabilities). Optimum cyanide production occurs during growth limitation (by oxygen), and with non-limiting amounts of iron. The producer organism is tolerant to cyanide. Bacterial HCN is generally regarded as a secondary metabolite, i.e. it does not seem to function directly in the processes of growth and development (primary metabolism). Cyanogenesis may have an ecological role and offer the producer a selective advantage (reviews by Castric, 1981; Blumer and Haas, 2000).

No reports exist of acute or chronic toxicity caused by bacterial cyanide in animals or plants. One reason for this is that the cyanogenesis is very tightly regulated in bacteria, such that local cyanide concentrations are usually below 1 mmol/l, a level that can be tolerated by many living cells. Only when massively infected by a cyanogenic pathogen, plants may suffer deleterious effects (Blumer and Haas, 2000 and references therein).

In *Chromatium* and fluorescent pseudomonads, glycine ($H_2N–CH_2–COOH$) is the immediate precursor of HCN; HCN and CO_2 are formed stoichiometrically by oxidative decarboxylation of glycine. Cyanide is derived from the methylene carbon (C2) of glycine and CO_2 from the carboxyl group (C1), as shown in experiments using radiolabelled [1-^{14}C]- and [2-^{14}C]-glycine in *C. violaceum*, *P. aeruginosa* and *P. fluorescens*. The C–N bond is retained during the reaction, excluding possible trans-amination or de-amination. HCN synthase or glycine dehydrogenase, the enzyme complex responsible for conversion of glycine into HCN and CO_2, is very labile and sensitive to O_2 that is required in limited amounts, i.e. micro-aerophilic conditions. Glycine stabilises HCN synthase ('training of bacteria'). Without glycine, only small amounts of HCN are produced. Iron and phosphate levels must be within optimal range, at different levels in *C. violaceum* and in *P. aeruginosa*, to allow HCN production to take place; the mechanism is not clear (on *C. violaceum*, see Rodgers and Knowles, 1978; Nazly *et al*, 1981; on pseudomonads, see Lorck, 1948; Freeman *et al*, 1975; Castric, 1977; reviews by Castric, 1981; Blumer and Haas, 2000).

HCN synthase may be a flavoprotein located on membranes; its topology suggests that HCN will be released into the periplasm rather than into the cytoplasm where it would have undesirable toxic effects (Blumer and Haas, 2000 and references therein).

A cluster of three genes, *hcnABC*, encodes HCN synthase, and its nucleotide sequence has been identified. In pseudomonads, HCN synthase is most active during the idiophase (transition from exponential to stationary phase), which is followed by a rapid decrease of HCN production. Anaerobic conditions prevent the loss of HCN synthase activity (Castric *et al*, 1979, 1981; Blumer and Haas, 2000 and references therein). Castric *et al* (1981) reported a maximum HCN formation of 12.2 nmol/15 min/10^9 cells in extracts from *P. aeruginosa*.

HCN is a good nucleophile and strong chelator of certain metal ions such as Ni^{2+}, Cu^{2+} and Co^{2+}, and acts as a potent inhibitor (by binding to the Cu_B^{2+} site) of cytochrome *c* oxidase (the terminal component of the respiratory chain in many organisms). In *P. aeruginosa*, an alternative, cyanide-insensitive terminal oxidase allows respiration and growth under cyanogenic conditions. HCN also inhibits several other metallo-enzymes like catalase, peroxidase, superoxide dismutase, nitrate reductase, nitrite reductase and nitrogenase. In *P. aeruginosa*, cyanogenesis is absent under denitrifying conditions, in the presence of nitrate and absence of O_2. Under N-limiting conditions, *P. fluorescens* can also detoxify external cyanide using α-ketoglutarate that instantly combines with cyanide, non-enzymatically, to form a less toxic cyanohydrin. By excreting copious amounts of α-ketoglutarate and pyruvate, *P. aeruginosa* can scavenge cyanide and utilise it as a nitrogen source (Blumer and Haas, 2000 and references therein).

Root-colonising strains of *P. fluorescens* protect several plants from fungal root diseases, e.g. tobacco black rot. Strains of *P. putida* seem to suppress certain disease symptoms such as leaf rust of wheat seedling leaves. *In vitro*, the inhibitory effect of cyanide occurs via the gas phase (Blumer and Haas, 2000 and references therein).

Wounds infected by *P. aeruginosa* contain cyanide, suggesting a possible role as a virulence factor (Blumer and Haas, 2000 and references therein).

Soil-dwelling *Pseudomonas* species grown under Fe-limiting conditions were able to mobilise CN from stable ferro- and ferri-cyanide complexes in soil, and formed a mixed iron-cyano complex (Blumer and Haas, 2000 and references therein).

In vitro, the blue-green freshwater alga *Anacystis nidulans* (intact cells) was able to produce HCN in the dark, from L-histidine (rather than D-histidine). The reaction required the presence of O_2 and cofactors such as peroxidase or metal ions. HCN formation increased, dependent on the histidine concentration, to a maximum of about 3 nmol HCN/l at 25 µmol histidine/45 µl cells/3 ml (8 mmol/l) and above after 3 hours (Pistorius *et al*, 1979) or 6.1 nmol HCN/3 ml/3 h = 0.68 µmol/l/h at 20 µmol histidine/l (Vennesland *et al*, 1981). Similarly, intact cells of *Plectonema boryanum* and the filamentous *Nostoc muscorum*, both common freshwater cyanobacteria, formed smaller amounts of cyanides from L- or D-histidine (maximum 0.26 and 1.34 nmol HCN/3 ml = 0.09 and 0.45 nmol/l) (Vennesland *et al*, 1981).

In some non-cyanogenic, anaerobic bacteria which oxidise or evolve hydrogen, cyanide occurs as an intrinsic ligand of NiFe- and Fe-hydrogenases. Examples are *Chromatium vinosum*, *Desulfovibrio vulgaris* and *D. desulfuricans*. The origin and physiological function of the cyanide ligand is unknown (Blumer and Haas, 2000 and references therein).

Fungi

HCN was first reported in the fruiting bodies of *Marasmius oreades* (common grassland disease causing 'fairy rings'), and was subsequently demonstrated in the sporophores of several other basidiomycetes (reviewed by Robbins *et al*, 1950). The mycelium of an unidentified basidiomycete isolated from white cedar severely inhibited the growth of various fungi incubated nearby (in the same culture cabinet). The fungicidal effect was caused by volatile HCN, probably from autolysis (Robbins *et al*, 1950).

Knowles (1976) quotes several sources identifying a large number of fungal species capable of producing cyanide. Cyanogenic strains exist among the following genera: *Clitocybe*, *Marasmius*, *Pholiota*, *Polyporus*, *Tricholoma*, *Basidiomycetes* and *Actinomycetes*. By way of example, two of the original papers reviewed and a further paper below, show that fungi may contain relatively high amounts of cyanide.

The mycelium of *M. oreades* produced HCN, probably by autolytic formation, to a maximum of 461 mg/kgdw when cultured on malt-yeast-glucose broth after 2 weeks. The optimum growth temperature was 20 to 25°C. HCN was also found *in situ* in the diseased portions of fairy rings; HCN was absent in the surrounding healthy turf (Lebeau and Hawn, 1963).

The 'snow mould fungus' (a psychrophilic basidiomycete causing winter crown rot of legumes and mould of grasses) also produces HCN in the autolysis stage (after some weeks). When cultured on alfalfa crown tissue in the laboratory, growing fungal mycelium contained 700 mg HCN/kgdw after 20 days (and even 2,500 mg/kgdw on soybean meal) at low temperatures. The concentration dropped off at 16 to 20°C. Such HCN concentrations were sufficiently high to kill buds and crown tissue of alfalfa plants (*Medicago sativa*) in western Canada (Lebeau and Dickson, 1953). HCN production during growth was stimulated by glycine alone, and almost 3 fold higher in combination with asparagine; the maximum production was 498.1 µg/kgdw after 7 days. Other amino acids were less effective. The cyanide carbon was shown to be derived from the C2 atom of glycine (from the added [2-^{14}C]-glycine rather than [1-^{14}C]-glycine) (Ward and Thorn, 1966).

In glucose-containing medium, the snow mould fungus produced HCN towards the end of the growth phase, from the methylene (radiolabelled) carbon of glycine; [2-^{14}C]-glycine, rather than [1-^{14}C]-glycine, was the preferred substrate. When cultured on acetate, HCN was derived from the methylene and carboxyl groups of glycine, and formed during the whole growth phase. In both cases, the maximum HCN content was reached after 2 weeks: up to 15 µmol/50 ml culture (Bunch and Knowles, 1980 as cited) or up to 10 µmol/50 ml culture, respectively. Some HCN was presumably lost due to volatilisation from the medium. Fungi grown on acetate and glycine were capable of utilising HCN (yielding CO_2) during growth (Bunch and Knowles, 1981).

Invertebrates

HCN may be formed from histidine in the hepatopancreas of mussels (*Mytilus edulis*) (Thoai *et al*, 1954 cited by Vennesland *et al*, 1981).

Cyanogenesis in arthropods, as far as is known (extensively reviewed by Duffey, 1981) occurs in 7 species of centipedes (*Chilopoda*), e.g. the soil dwelling *Pachymerium ferrugineum* and some varieties of the garden centipede *Geophilus*, 46 species of millipedes (*Diplopoda*) like the greenhouse millipede *Oxidus gracilis* and several varieties of polydesmids (e.g. *Polydesmus angustus*). It also occurs in 10 species of insects (*Insecta*), the latter including 3 beetles, 4 moths and 3 butterflies. The enzymology of insect cyanogenesis is largely unknown, as are the ultimate cyanogens. Several nitrile (–C≡N) containing compounds have been related to cyanide production in different insects, for example: benzoyl cyanide, mandelonitrile, mandelonitrile benzoate, and the glucosides linamarin and lotaustralin, but only the latter three compounds have been unambiguously confirmed (Duffey, 1981 and references therein).

The cyanide content of insects can be very high, for example in gravid six-spotted burnet moths (*Zygaena filipendulae*), 370 - 460 µg HCN/adult (66 - 299 mgbw) was present as linamarin and lotaustralin, primarily in the eggs. The polydesmid *Harpaphe haydeniana* (yellow-spotted millipede) contained R-mandelonitrile equivalent to about 8 µg HCN/animal, out of a total of 16 µg HCN/animal (250 mgbw). Mandelonitrile is also probably the major cyanogen in other millipedes. The centipedes *Asanada* species (35 mgbw) and *Pachymaerium ferrugineum* (bw not stated) contained the equivalent of 6.6 and 2.2 µg HCN/animal, respectively (Duffey, 1981 and references therein).

Cyanogenic secretions usually contain a mixture of HCN and mostly benzaldehyde, but also several other substances like mandelonitrile, mandelonitrile benzoate, benzoic acid, benzoyl cyanide, phenol guaiacol, and fatty acids. The moth *Zygaena filipendulae* was able to release 200 µg HCN per time. A certain polysdesmid, *Pseudopolydesmus branneri*, released 32 µg HCN/time (199 mgbw), two xystodesmid *Apheloria* varieties 16 and 27 mg µg HCN/time (769 and 1,050 mgbw) and *Apheloria corrugata* variable amounts up to 600 µg HCN/time (400 - 1,400 mgbw) (Duffey, 1981 and references therein).

Biosynthesis of HCN *de novo* from exogenous L-phenylalanine via mandelonitrile, with by-products benzaldehyde and benzoyl cyanide, was demonstrated in several millipedes, using radiolabelled ^{14}C- and ^{3}H-precursors. The cyanogenic glands of *H. haydeniana* contain the corresponding hydrolases, β-glucosidase and α-hydroxynitrile lyase, which together allow high *in vivo* production of HCN. The pathway and enzymes also occur in plants. *H. haydeniana* stores large oily droplets of mandelonitrile in a chamber, to which glucosidase is added; the labile mixture is then entered (as a plug from the nitrile droplet) as needed (controlled by a muscle) into

a separate reaction chamber already filled with α-hydroxynitrile. Upon reaction, the gaseous products are emitted through a hole in the cuticle. Each phalange has a multi-cellular cyanogenic gland in the distal portion. *H. haydeniana* can 'fire' individual cyanogenic glands, singly or in unison, depending on where the animal is physically stimulated. Most polydesmoid millipedes possess similar capabilities, and secrete the cyanogenic fluid in an oozing way, but the xystodesmid *Cleptoria rileyi* can squirt jets of cyanogenic fluid up to a distance of several centimetres. Repetitive physical stimulation inhibits firing (Duffey, 1981 and references therein).

In zygaenids, the cyanogens (linamarin and lotaustralin) appear to be generally distributed in the body fluids or tissues, and crushing the insect liberates HCN. The mechanism of spatial separation of glucosidase and a putative nitrile lyase is unknown (Duffey, 1981 and references therein).

In the scolopendrid centipede *Asanada* species, numerous unicellular glands are spread all over the body (apart from the first and last body-leg segments). Each gland has a single pore opening to the external cuticle and contains two different secretions from an unknown cyanogenic precursor; there are about 300 pores per segment. HCN is released when the two secretions mix. Proteins are detectable in the secreted mixture (Duffey, 1981 and references therein).

Ecologically, it is believed that cyanogenic secretion by millipedes serves as a deterrent against predators. However, there is little, or even contradictory evidence from field studies. For example certain phengodid beetle larvae are predators of both cyanogenic and benzoquinone producing millipedes. Another possibility is that cyanogenesis (in combination with other cyanogenic chemicals) contributes to the aseptic status of the litter-dwelling millipedes' cuticle, and keeps it free from undesirable micro-organisms (Duffey, 1981).

Mammals (leukocytes)

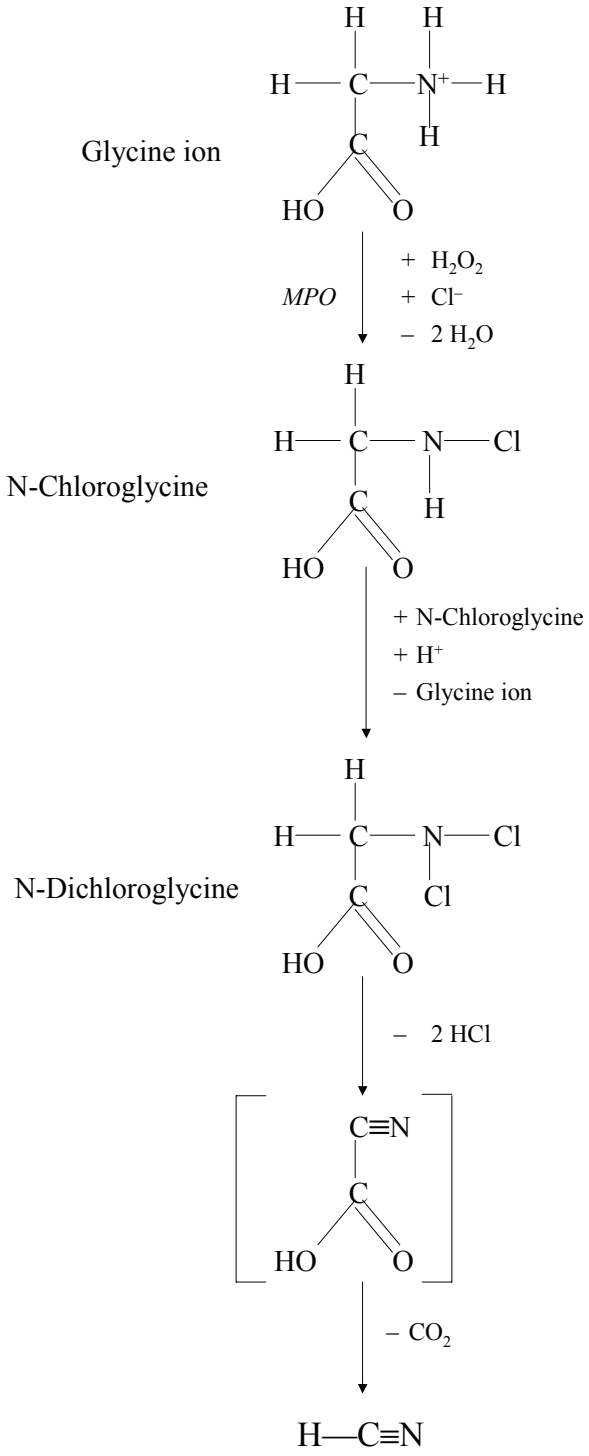
Cyanide is produced during phagocytosis as a bactericide. In leukocytes, cyanide is generated by a myeloperoxidase (MPO)-mediated reaction in which oxidative chlorination of glycine or N-terminal glycyl peptide as well as aromatic amino acids occurs, and by oxidation of thiocyanate by peroxidase enzymes (Chung and Wood, 1970; Zgliczinski and Stelmaszynska, 1979; Stelmaszynska and Zgliczinski, 1981; all cited by Borowitz *et al*, 1997).

During phagocytosis, MPO is discharged into the phagocytic vacuole and accumulates on the surface of the invading bacteria, thereby inducing enzymatic chlorination (Cl is abundant in animal bodies) and hydrogen peroxide formation. N-Dichloroglycine, the chlorinated product from glycine, and chlorinated N-terminal glycine residues of peptides and proteins, yield HCN. During phagocytosis of *Staphylococcus epidermidis* in blood obtained from healthy volunteers,

levels were up to 5.9 nmol HCN/10^7 leukocytes after 0.5 to 1 hour of incubation *in vitro*. Phagocytosis of *Escherichia coli* yielded less, up to 2.6 nmol HCN/10^7 leukocytes after 45 minutes. HCN in turn may act on bacterial haem-proteins and/or diffuse to the cytosol and inhibit catalase activity

. It is speculated that HCN excreted in trace amounts in urine and expired air of normal humans, stems, at least in part, from the ongoing defence process in tissues (Stelmaszynska and Zgliczinski, 1981) (Figure 8).

Figure 8: Chlorination of glycine as a source of HCN (Adapted from Stelmaszynska and Zgliczinski, 1981)



[ ] = Hypothetical intermediate

Phagocytosis by polymorphonuclear leukocytes (PMNs) and all post-phagocytic events occurring within the PMNs represent one of the lines of resistance of the human body against invading micro-organisms. The term 'phagocytosis' denotes the act of engulfment of bacteria. Post-phagocytic events include: morphologic changes, a burst of leukocytic metabolic activity, and the killing and digestion of ingested micro-organisms (reviewed by Klebanoff, 1975 cited by Stelmaszynska and Zglizcynski, 1981). The killing of micro-organisms is accomplished by mechanisms that are partly oxygen-dependent and partly oxygen-independent. MPO is an important component of the latter mechanism (Klebanoff, 1975 cited by Stelmaszynska and Zglizcynski, 1981).

4.1.4 Other sources

Car exhaust

Cyanides have been detected in automobile exhaust. The average emission rate was 12 mg HCN/mile [a] (7.5 mg/km) (General Motors, 1975 cited by Fiksel *et al*, 1981). On this basis, the latter author calculated a total emission of 18 kt HCN/y for the whole US car fleet (Fiksel *et al*, 1981). Another source estimated a fleet average emission of 7.1 mg HCN/km (Harvey *et al*, 1984). These data are over 30 years old, and only for the US market, and relate to cars with and without catalytic converters.

The Task Force has estimated approximately 6.1 kt of HCN presently emitted by light vehicles world-wide. This figure was calculated as follows: there are currently more than 531×10^6 passenger cars world-wide (Worldwatch, 2004). In the USA in 1993, the average car travelled 11,400 miles/y, increasing by approximately 3% between 1988 and 1993, the largest part of that increase and a more than proportional share in miles/year in the segments 'minivans and light trucks' (EIA, 2004). At that time (1993), there were 156×10^6 passenger cars in the US, travelling 1.793×10^{12} miles in one year. An update survey (no full report) of EIA in 2003 indicates 2.3×10^{12} miles and 202×10^6 cars (EIA, 2004). Based on these figures and an estimated emission of 1 mg HCN/mile (with catalytic converter), approximately 2.3 kt HCN may be discharged by vehicles (excluding heavy trucks). Extrapolated to 531×10^6 cars worldwide, this would correspond to approximately 6.1 kt HCN emitted world-wide by light vehicles.

Emissions from jet aircraft appeared to be insignificant (Cicerone and Zellner, 1983).

[a] 1 mile = 1.61 km

Accidental fires

A number of synthesised (e.g. polyacrylonitrile, polyurethane, polyamide, urea-formaldehyde, melamine) and natural (e.g. wool, silk) compounds produce HCN when burned. These combustion gases likely contribute to the morbidity and mortality of smoke inhalation (Savolainen and Kirchner, 1998).

In addition to CO, much HCN is released during fires. This might affect fire-fighters inhaling smoke. The impact of cyanide during accidental fires is not fully understood.

Volcanoes and lightning

HCN can be released from volcanoes, especially in high temperature low-oxygen zones. HCN formation from CN radicals due to lightning has been considered possible but insignificant as a source (Cicerone and Zellner, 1983).

4.2 Environmental distribution

For the partitioning of cyanides in the environment, only the distribution of HCN is relevant. NaCN, KCN and ACH all hydrolyse and release HCN, which is the substance of concern (Section 2.3, Chapter 7 introduction).

The theoretical distribution of HCN in a 'standard' environment was calculated following the fugacity-based equilibrium criteria (EQC) level I and II models (Mackay *et al*, 1996) and using the basic physico-chemical properties given in Table 1 (Section 2.3.1). The level I and II models assume that a steady-state equilibrium partitioning (in a closed evaluative environment) is achieved without resistance to mass transfer between the environmental compartments (air, water, soil and sediment). This means that irrespective of the input compartment and the rate of degradation in the environmental compartments, the concentration ratios between the compartments are considered always to be controlled by the partitioning between the compartments and remain constant in time (Table 7).

Table 7: Distribution of HCN following Mackay model, level I and II (ten Berge, 2004a)

Input parameter	**Value, unit**
Temperature	25°C
Molecular mass	27.0
Vapour pressure	976 hPa
Dimensionless Henry's Law coefficient air-water	0.0054
Water solubility	687 g/l [b]
Log K_{ow}	–0.25
Compartment	**(%)**
Air	73.1
Water	26.9
Soil	0.0134
Sediment	2.97×10^{-4}
Suspended solids	9.30×10^{-6}
Aquatic biota	7.56×10^{-7}

[b] Represents relative density because of infinite water solubility

4.3 Environmental fate and biotransformation

This section reviews the atmospheric, aquatic and terrestrial fate of HCN (and ACH) in the environment and in biological sewage treatment plants. It also discusses the potential for bioaccumulation.

4.3.1 Atmospheric fate and impact of hydrogen cyanide

This section reviews the chemical and physical removal processes of HCN and its potential for ozone depletion, global warming and tropospheric ozone formation, and possible contribution to the acidity of rainwater. The degradation mechanism and products of HCN are discussed. The expected atmospheric fate of ACH is also briefly discussed. A historical perspective is given.

Chemical and physical removal processes

Most airborne cyanide exists as HCN gas, although small amounts of metal cyanides may be present as particulate matter (Graedel, 1978 cited by US-EPA, 1984). Metal cyanide particles,

particularly water-soluble cyanide particles, are expected to be removed from the air by both wet and dry deposition. Their contribution to total cyanide removal is not well understood (ATSDR, 1998).

HCN reacts with naturally occurring hydroxyl radicals (˙OH) formed by sunlight through addition on the double bond, followed by rapid oxidation to CO and nitric oxide (NO) (see below). This photo-oxidation occurs both in the troposphere (0 - 8 km) and stratosphere (up to 80 km). Another path for HCN oxidation, dominant in the higher stratosphere (> 34 km), was reportedly the reaction with singlet oxygen O(^1D). Direct photolysis of HCN was assumed to play a minor role at high altitudes (> 54 km). About 98% of HCN (from ground-level sources) was considered to remain in the stratosphere. In all, tropospheric oxidation by ˙OH addition presents the major sink of HCN (and a source of NO_x, see below). Reactions with other gases such as CO or O_3 do not occur (Cicerone and Zellner, 1983).

HCN is not very soluble in water at low partial pressures (< 1 torr = 1 mm Hg = 1.33322 hPa). The Henry's Law constant was 4,000 torr/mol/mol at 18°C and 0.01 atm (10.13 hPa). The Henry coefficient was also defined by the Ostwald solubility coefficient of about 252 (= 106 mol/m^3) at a pressure of 10.13 hPa (Cicerone and Zellner, 1983). These values are equivalent to a Henry constant of 9.56 Pa•m^3/mol or a dimensionless ratio of the concentration in air/water of 0.0040 at 18°C. The dimensionless Henry constant is increased to 0.0054 at 25°C due to the higher vapour pressure at that temperature (Table 1, Section 2.3.1).

Because of its low solubility and the weak acidity ($K = 1.3 \times 10^{-9}$), HCN was expected to have a very long atmospheric lifetime against rainout. Thus, it would take 34 years of average rainfall (1 m/y) to remove 200 ppt of gaseous HCN (cf. Section 5.1.1: background concentration of 166 ppt) from the atmosphere if the rain were saturated with HCN at pH 4. Owing to its low water solubility and weak acidity, and to its photochemical inertness, there should be no significant diurnal variation in HCN concentrations, and there were "no obvious and major roles" for HCN in tropospheric chemistry (Cicerone and Zellner, 1983). This has been the conventionally held view until the 90s.

It was noted that the reaction of HCN with OH radicals can only remove a small part of the large input of HCN from biomass burning. Crutzen and Andreae (1990) contrasted a calculated atmospheric HCN input of 0.33 to 1.13 Mt N/y (1.0 - 2.0 Mt HCN/y) from biomass burning with 0.2 Mt being removed by OH radicals (Lobert, 1990 as cited). These, and other, authors proposed the existence of other major sinks such as take-up by the oceans or consumption by vegetation (nitrogen-fixation).

Li *et al* (2000), in order to explain the large seasonal variations in (global) HCN concentrations seen by other authors (below), proposed that the Henry's Law constant was sufficiently high

(7.5 - 12 mol/atm at 25°C; Hine and Weimar, 1965; Edwards *et al*, 1978; Gaffney *et al*, 1987; all as cited) for ocean uptake to provide the main (global) sink. (This value of Henry's Law constant is equivalent to 7,500 - 12,000 mol/m^3/atm; in this report, the reciprocal value is used 8.33×10^{-5} to 1.33×10^{-4} atm·m^3/mol [8.17 - 13.04 Pa·m^3/mol] or the dimensionless ratio air/water of 0.0034 to 0.0055.) The deposition velocity for HCN was 0.13 cm/s. The authors suggested that HCN, once dissolved in seawater (pKa = 9.2 at 25°C), might be consumed biologically or chemically in the ocean (by an unknown mechanism) on a time scale of a few months or less (see below).

Further support for the presence of an additional sink in the marine boundary layer has been obtained from an atmospheric model analysis based on acetonitrile (CH_3CN) data (De Latt *et al*, 2001 cited by Wisthaler *et al*, 2002). These data were collected during the 1999 Indian Ocean experiment (INDOEX) campaign to characterise the extent and chemical composition of contaminated outflow from the Indian subcontinent during the dry winter season. The ocean sink hypothesis for HCN (and CH_3CN), however, remained unconfirmed (Wisthaler *et al*, 2002).

An analysis of vertical HCN (and CH_3CN) profiles measured in the northern Pacific troposphere (0 - 12 km) in 2001 (TRACE-P, Section 4.1.1) showed reduced concentrations in the marine boundary layer (the actual profiles are presented in Section 5.1.1). This was considered as a first indication of a surface sink (Singh *et al*, 2003). Using a simple steady-state box model (Singh *et al*, 1996 as cited), the observed median gradient of 47 ppt HCN across the top of the marine boundary layer (2 - 4 km) (Table 16, Section 5.1.1) was used to derive a net oceanic deposition (loss) of 8.8×10^{-15} g N/cm^2/s for HCN, corresponding to a mean deposition velocity of 0.12 cm/s over the open ocean. The air-sea exchange model of Liss and Slater (1974 as cited), newly parameterised from several recent references (as detailed by the authors [Singh *et al*, 2003]), was then used to estimate an under-saturation of 27% (± 20%), needed to maintain the calculated oceanic (net) deposition flux of 1.0 Mt N/y of HCN, thus contributing a small fraction of the total global nitrogen (nutrient) input into the world oceans (estimated at 30 Mt N/y by Duce, 1998 as cited). The authors suggest the possibility of higher HCN deposition velocities over coastal regions (with upwelling currents). Aerobic biological, rather than chemical, degradation was believed to be the likely mechanism of oceanic removal, needed to maintain permanent HCN under-saturation of seawater (Singh *et al*, 2003).

Li *et al* (2003) confirmed the ocean sink hypothesis by means of more sophisticated atmospheric modelling (Li *et al*, 2000) of the TRACE-P profiles observed by Singh *et al* (2003), as explained below**.**

Atmospheric lifetime [a]

Cicerone and Zellner (1983) developed a numerically iterative model that integrated the total atmospheric flux of HCN from reaction with ˙OH, singlet oxygen O(^1D) and direct photolysis. Input parameters were taken from earlier work of the same and other authors. The effective rate constant for OH addition decreased with rising altitude (˙OH concentration): K_{OH} ranged from 0.022 to 0.011×10^{-12} cm^3/molecule/s in the troposphere ($3.20 - 8.60 \times 10^6$ ˙OH/cm^3), and 0.0088 to $0.0000069 \times 10^{-12}$ cm^3/molecule/s in the stratosphere ($21.3 - 4.81 \times 10^6$ ˙OH/cm^3, maximum 32.0×10^6 ˙OH/cm^3). The rate constant for reaction with stratospheric O(^1D) was set at 10^{-12} cm^3/molecule/s, independent of pressure and temperature (altitude). HCN photoabsorption and dissociation were assumed to be similar to those of HCl, and parameterised from other work by the same authors. The resulting overall atmospheric lifetime for HCN was estimated to be 2.5 years, or between 1.3 and 5.0 years when ˙OH concentrations were doubled or halved, respectively.

Li *et al* (2000) extended the above model by adding ocean uptake flux as a major sink for atmospheric HCN. The objective was to simulate the amplitude of seasonal variations in HCN concentration at northern mid-latitudes observed by other authors. For example, Mahieu *et al* (1995, 1997 as cited) and Zhao *et al* (2000 as cited) observed a 2 to 3 fold variation with maxima in spring-summer using atmospheric infra-red column measurements; Rinsland *et al* (1998 as cited), using space-based infra-red measurements, found HCN concentrations in the tropical upper troposphere to be 5 fold higher during the dry season.

In the Li *et al* (2000) model, biomass burning was, following various authors cited, taken as the only (global) source of atmospheric HCN. Other, unquantified, sources were neglected, including higher plants and fungi, and, in the stratosphere, ion-catalysed CH_3CN conversion. The magnitude of this source was 2.9 Mt N/y, calculated from a biomass burning CO inventory (Wang *et al*, 1998 as cited), assuming a maximum molar emission ratio of 1.1% compared to CO (Lobert *et al*, 1990, 1991 as cited). The initial HCN concentration was uniformly set at 170 ppt (cf. Section 5.1.1: background concentration measured from Kitt Peak: 166 ppt between 2.1 and 12 km [Rinsland *et al*, 1982 cited by Cicerone and Zellner, 1983]).

[a] Lifetime is the time necessary for 63% degradation; it is equal to the 'half-life' divided by ln 2 (= 0.693)

Ocean uptake of HCN from the marine boundary layer was modelled by Li *et al* (2000) following the formula of Liss and Slater (1974 as cited):

$$F = k_w C_g K_H RT \quad \text{..... (Eq. 12)}$$

Where F = flux

k_w = air-to sea transfer velocity (m/s), calculated from wind speed at 10 m height, diffusivity and kinematic viscosity of water (details given by Li *et al*, 2000)

C_g = concentration of HCN in surface air

K_H = Henry's Law constant (temperature dependent), 12 mol/atm at 25°C (13.04 Pa·m^3/mol)

R = universal gas-phase constant

T = temperature (K)

Additional sinks for atmospheric HCN were specified by Li *et al* (2000) following Cicerone and Zellner (1983), assuming for the photo-oxidation reaction temperature-dependent values of $K_{OH} = 0.12 \times 10^{-12\ (-400/T)}$ to $0.15 \times 10^{-30\ (-875/T)}$ cm^3/molecule/s for the high- and low-pressure (10 hPa) limits of the model, respectively. Monthly varying ˙OH concentrations in the troposphere were taken from Bey *et al* (1999 as cited) and stratospheric (monthly varying) ˙OH and O(^1D) concentrations data from Schneider *et al* (1999 as cited). The rate constants for the reaction with stratospheric O(^1D) and HCN photolysis were adopted from Cicerone and Zellner (1983) (above). The model was driven by meteorological data collected in 1993 and 1994 by the Goddard earth observing system (GEOS) data assimilation system; the data were updated every 6 hours by GEOS. In a sensitivity analysis, HCN emission was assumed to be 53% less and the transfer velocity k_w was reduced by a factor 6 (to describe that 5/6 of HCN taken up by the ocean was assumed to be returned to the atmosphere). The outcome of the model was an overall atmospheric lifetime for HCN of 2.1 to 4.4 months (0.18 - 0.37 y) for the standard and sensitivity simulation, respectively (Li *et al*, 2000).

Similarly, but using different assumptions, Singh *et al* (2003) calculated an atmospheric lifetime of for HCN of 5.3 months based on an ocean removal rate of 1.0 Mt N/y (above), and 63 months for removal by reaction with ˙OH radicals ($K_{OH} = 0.12 \times 10^{-12\ (-773/T)}$) assuming a global mean ˙OH concentration of about 1×10^6 molecules/cm^3. Rainout and washout were expected to be unimportant, due to the low solubility of HCN (Crutzen and Lawrence, 2000 as cited). Removal by reaction with Cl, O(^1D) and NO_3 radicals was calculated to be negligible. Soil degradation of HCN, upon permanent deposition, was expected to be minor. The mean overall atmospheric lifetime, based on removal by ˙OH and oceanic loss, was 5.0 months (0.42 year) for HCN (Singh *et al*, 2003).

Li *et al* (2003) adapted their model (Li *et al*, 2000 above) to simulate the vertical gradients of HCN (and CH_3CN and CO) observed during TRACE-P in 2001 (Singh *et al*, 2003; actual profiles are given in Section 5.1.1). Biomass burning and residential coal burning emissions from south east Asia (identified by the molar HCN:CO ratio of 0.27%) were the main sources of HCN and provided the best fit for simulation of the observed TRACE-P vertical profiles. Ocean uptake represented the dominant sink and oxidation by ˙OH an additional minor sink. Using a deposition velocity of 0.13 cm/s and a Henry's constant of 0.0034 (air/water) (8.17 Pa•m^3/mol), the resulting tropospheric lifetime for ocean uptake of HCN was 3 months. The lifetime for oxidation by tropospheric ˙OH alone was 4.3 years. Thus, the overall tropospheric lifetime was 5.3 months (0.44 year), and the overall atmospheric lifetime 6.2 months (0.52 year).

Earlier values to describe the atmospheric lifetime of HCN have been reported, but these are all limited by assuming (tropospheric) photo-oxidation to be the main sink.

Graedel (1978 cited by US-EPA, 1984) reported a half-life of approximately 11 years, assuming $K_{OH} = 0.002 \times 10^{-12}$ cm^3/molecule/s, also based on a concentration of 1×10^6 ˙OH/cm^3.

Fritz (1982) measured the rate constant oxidation for ˙OH addition in the laboratory. The value was $K_{OH} = 0.03 \times 10^{-12}$ cm^3/molecule/s at an initial ˙OH concentration of 1×10^6 molecule/cm^3, considered to be representative of tropospheric conditions (smog). The resulting lifetime for HCN was in the order of 1 year.

Fiksel *et al* (1981) extrapolated K_{OH} from reactions with similar molecules and proposed a maximum value of 0.1×10^{-12} cm^3/molecule/s. The authors, following Eschenroeder *et al* (1978 as cited), suggested a lifetime of HCN between one month in urban (polluted) air and a year in rural (background) air.

Based on $K_{OH} = 0.03 \times 10^{-12}$ cm^3/molecule/s (Fritz *et al*, 1982) and assuming an average concentration of 0.5×10^6 ˙OH/cm^3, the lifetime of HCN was about 2 years (ATSDR, 1998).

Howard *et al* (1991) calculated tropospheric half-lives of 89.1 days (0.25 y) to 2.4 years for photo-oxidation of HCN in polluted (30×10^6 ˙OH/cm^3) and background (3×10^6 ˙OH/cm^3) air, respectively, according to the original method of Atkinson (1987).

Using Atmospheric Oxydation Program software from SRC (2003), the estimated half-life was 356.5 days (1 y), assuming $K_{OH} = 0.03 \times 10^{-12}$ cm^3/molecule/s based on 1.5×10^6 ˙OH/cm^3 during a 12-hour day. The software is based on the improved method of Atkinson (Meylan and Howard, 1993).

Table 8 summarises the available lifetimes and corresponding half-lives (or vice versa).

Table 8: Atmospheric lifetimes and half-lives of HCN (by decreasing values)

OH concentration (10^6 molecule/cm^3)	K_{OH} [a] (10^{-12}cm^3/molecule/s)	Half-life (y)	Lifetime (y)	Reference
Photo-oxidation only				
1	0.002	11	16	Graedel, 1978 [b]
Not stated [c]	$0.009 - 0.0074 \times 10^{-18}$ [d,e]	3.0	4.3	Li *et al*, 2003
3	Not stated	2.4	3.5	Howard *et al*, 1991
3.20 - 8.60, 21.3 - 4.81, maximum 32.0 [f]	0.022 - 0.011, $0.0088 - 0.0069 \times 10^{-3}$ [d]	1.7 (0.9 - 3.5)	2.5 (1.3 - 5.0)	Cicerone and Zellner, 1983
0.5	0.03	1.4	2	ATSDR, 1998
1.5	0.03	1	1.4	SRC, 2003
1	0.03	0.7	1	Fritz *et al*, 1982
30	Not stated	0.25	0.36	Howard *et al*, 1991
Not stated	0.1	0.06 - 0.7	0.08 – 1	Fiksel *et al*, 1981
Added oceanic uptake				
Not stated [c]	$0.009 - 0.0074 \times 10^{-18}$ [d,e]	0.30 - 0.36	0.44 - 0.52	Li *et al*, 2003
1.5	0.1×10^{-12} [d,g]	0.29	0.42	Singh *et al*, 2003
Not stated [h,i]	$0.12 - 0.15 \times 10^{-18}$ [d]	0.12 - 0.25	0.18 - 0.37	Li *et al*, 2000

[a] Usually at 25°C (lower troposphere), if not stated otherwise
[b] Cited by US-EPA, 1984, p.1
[c] Monthly mean ˙OH concentrations from Li *et al*, 2002 (as cited)
[d] Temperature dependent; ranges apply to low and high altitude (high - low pressure)
[e] Data from Wine *et al*, 2002 (as cited)
[f] Data from Cicerone *et al*, 1983 and Thompson and Cicerone, 1982 (both as cited)
[g] Data from Wine *et al*, 2002 (as cited) at 600 torr (as cited)
[h] Tropospheric data from Bey *et al*, 1999 (as cited)
[i] Stratospheric data from Schneider *et al*, 1999 (as cited)

Ozone depleting potential [a]

HCN itself does not react with O_3 (Cicerone and Zellner, 1983).

[a] Ozone depleting potential expresses the stratospheric ozone loss due to emission of a unit mass of a given compound, divided by the ozone loss due to emission of the same mass of a reference compound.

Global warming potential [a]

HCN itself has "no significant role in climate" due to overlaps with other major infra-red absorbers in the atmosphere (Ramanatan and Kiehl, 1982 personal communication cited by Cicerone and Zellner, 1983).

Tropospheric ozone formation

HCN does not contribute to O_3 formation in the troposphere (see below).

Contribution to the acidity of rainwater

No data are available.

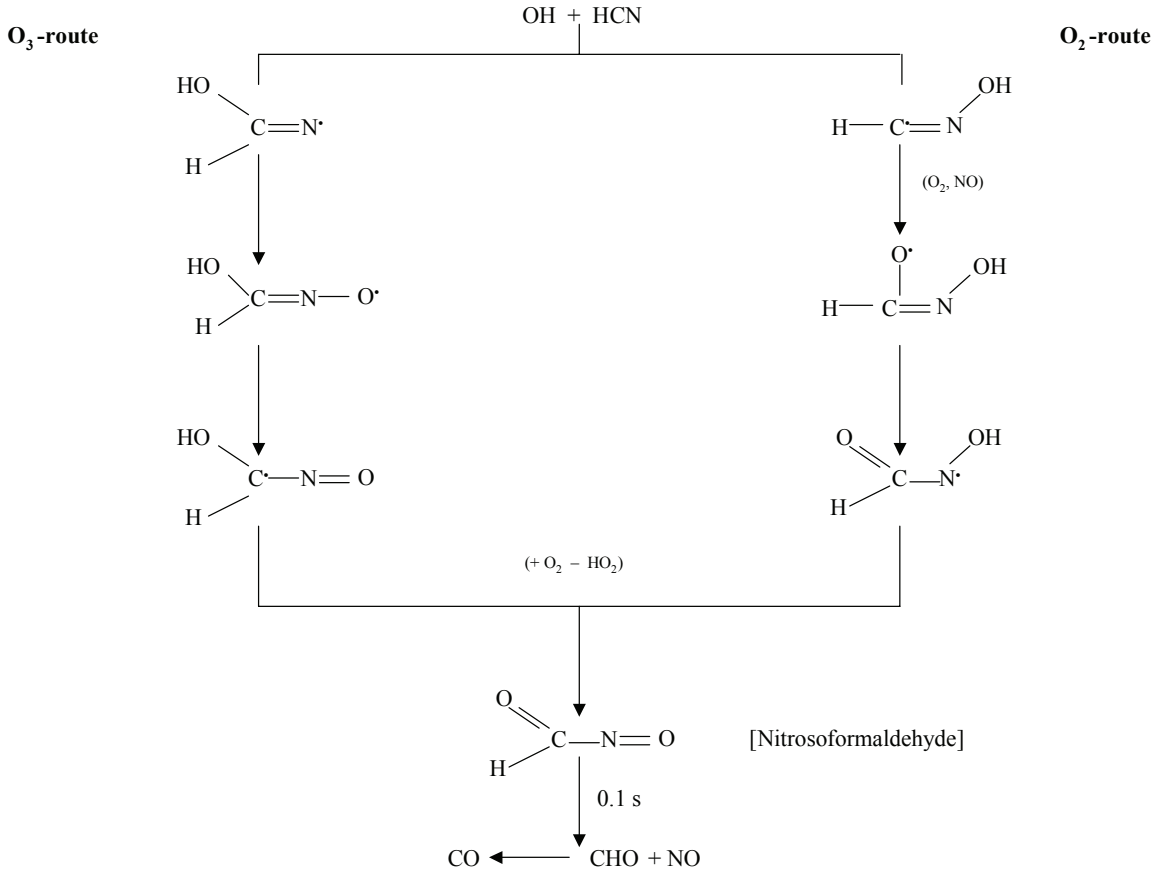
Degradation mechanism and products

The HCN molecule is strongly bound; it presents a stable gas in the atmosphere (Crutzen *et al*, 1979 cited by Cicerone and Zellner, 1983). The large enthalpies exclude many theoretically possible endo/exothermic reactions in the gas phase; only those with ˙OH and O(^1D) are important. Photodissociation is only expected in the upper stratosphere (UV light, λ = 175 - 238 nm).

Fritz *et al* (1982, 1983 cited by Cicerone and Zellner, 1983) studied the reaction of HCN and ˙OH in the laboratory, where a fairly stable (for a few milliseconds) HCNOH adduct was formed. The one H˙CNOH isomer would then combine with O_2 or NO, and the other HC˙NOH isomer with O_3, either way yielding nitrosoformaldehyde (HOCNO) that further degrades to CHO and NO. Overall, these reactions occur within minutes. CHO then quickly reacts with O_2 to give CO and HO_2. Final degradation products of HCN are thus CO and NO, and the CN bond is permanently split (Figure 9).

[a] Global Warming Potentials (GWPs) express the radiative forcing (increase in earthward infra-red radiation flux) due to emission of a unit mass of a given compound, divided by the radiative forcing due to emission of the same mass of a reference compound.

Figure 9: Tropospheric degradation of HCN (Fritz *et al*, 1982)



The scheme was further elaborated by Cicerone and Zellner (1983) for the purpose of their degradation model. (The latter authors postulate another, less likely O_2 route for transformation of HC˙NOH to HOCN and HO_2.)

In the stratosphere, both photodissociation and reaction with O(^1D) will yield CN radicals, which probably quickly combine with O_2 to form NCO. Competition with H-donors (R-H) is unlikely and reformation of HCN can be excluded (Cicerone and Zellner, 1983).

Atmospheric fate of ACH

Due to its very low vapour pressure (4 hPa, Table 4), ACH will not exert an extensive vapour above the liquid phase. Commercial ACH does, however, contain approximately 0.05% HCN (by weight) in equilibrium (Section 2.3) and this, due to its relatively high vapour pressure, will predominate in the vapour phase. ACH vapour is unstabilised (as opposed to liquid ACH which is

acid stabilised) and will rapidly break down to HCN and acetone. ACH has not been measured in the environment.

Chemical and physical removal processes

ACH is predicted to be susceptible to photo-oxidation in air (Atkinson, 1987).

Evaluation of atmospheric fate

The conventional view that photo-oxidation is the main degradation reaction of HCN has been challenged by modelling results and airborne observations that pinpoint biomass burning as the main source and ocean uptake as the main sink, on a global scale.

(Tropospheric) photodegradation proceeds fairly slowly (faster in polluted air with higher ˙OH concentration), with a lifetime of about one to a few years. Possible reactions with ozone and singlet oxygen in the higher atmosphere are insignificant. The final HCN breakdown products are CO and NO. Removal by wet deposition (rainout) does not seem to play a role.

In contrast, ocean uptake, caused by permanent under-saturation of seawater maintained by an unknown (presumably biological, Section 4.3.4) process, is a relatively quick process with a lifetime of a few months. Thus, the overall lifetime of HCN has been estimated to be 5.3 months in the troposphere, and 5.0 to 6.2 months in the whole atmosphere.

As a consequence, on a global scale, HCN would not have the potential to be transported over long distances before being removed by physical or chemical processes.

ACH is susceptible to photo-oxidation, with an estimated lifetime of a few months. The degradation mechanism is unknown.

NaCN and KCN are expected to be removed by wet and dry deposition.

No particular impact on ozone depletion, global warming, and tropospheric ozone or acid rain formation is expected for any of these cyanides.

4.3.2 Aquatic fate

A number of processes contribute to the aquatic fate of readily dissociable cyanides such as NaCN and KCN, and HCN: the pH- and temperature-dependent dissociation of HCN, volatilisation of HCN from the water body, formation of complex cyanides in the presence of transition metals, and photolytic degradation of those cyanide complexes. The following may contribute to a lesser extent: photolytic oxidation of CN^- to cyanate (OCN^-), abiotic degradation of cyanides, adsorption to sediment and particulate matter, and biodegradation by a variety of organisms (Section 4.3.4).

ACH has been reported to dissociate freely in freshwater and seawater into HCN and acetone. The dissociation process is uni-molecular and only influenced by temperature and concentration. A 0.1% solution of ACH has been determined to achieve 84 to 85% dissociation at equilibrium. As the dilution of ACH approaches infinite, the association rate nears zero and hence in dilute form ACH will effectively fully dissociate. The half-life for dissociation of ACH to acetone and HCN is pH dependent; it has been calculated, for a 0.1% solution, as 57 minutes (pH 4.9), 28 minutes (pH 6.3) and 7.8 minutes (pH 6.8) (Hobson, 1993; Rohm and Haas, 1986).

Measurements of the decomposition of ACH dissolved in heavy water (D_2O), using a non-invasive infra-red absorption method of analysis ('attenuated total reflection Fourier transform infra-red spectroscopy'), have confirmed the findings of the earlier studies. The half-life of a dilute solution of ACH (2,000 mg/l) above pH 7.0 was shown to be less than 5 minutes. Furthermore, extrapolation of the data to single ppm concentrations confirmed that ACH will decompose rapidly and quantitatively to HCN and acetone (Frank *et al*, 2002). Based on the rapid hydrolysis of ACH to HCN and acetone, the considerations for the aquatic fate of cyanides are relevant for ACH as well.

Dissociation equilibrium and volatilisation of HCN

The dissociation of HCN depends on temperature (Table 1) and pH. Typical pH values of natural waters range from pH 6 to 8. The pKa value of the dissociation equilibrium of $HCN \rightleftarrows H^+ + CN^-$ is 9.36 at 20°C (Table 1). This implies that in simple cyanide solutions, 50% of the cyanide will be present as HCN at pH 9.36 (Broderius, 1973). The HCN fraction will be 92% at pH 8.5 and > 99% at pH < 7 (Leduc *et al*, 1982). In a model hard water, 98% of free cyanide was HCN at pH 7.7 (Murgatroyd *et al*, 1998).

Given its high vapour pressure (Table 1), HCN is expected to volatilise from water bodies into the air. The rate of volatilisation will depend on the temperature and wind speed. Callahan *et al* (1979) reported a number of unpublished experiments to elucidate the volatilisation half-life of

cyanide under different conditions. The volatilisation half-life from a natural water sample (8 l), spiked with KCN (25 - 200 µg CN^-/l) and standing uncovered in the laboratory without wind influence, was 22 to 111 hours (4.6 d). Outside the laboratory, in open air with moderate wind velocities, the evaporation increased by a factor of 2 to 2.5. In another experiment, air (flow 2 cm^3/min) was passed through a solution (6 l) containing 10 mg CN^-/l at pH 7 and 30°C. After 375 minutes (6.25 h), about 42% of the cyanide had volatilised (Raef *et al*, 1977a,b).

If the volatilisation rate of HCN needs to be estimated in a local situation, it appears important to define the temperature, atmospheric pressure (altitude), extent of stirring of the water phase, wind speed and the ratio of surface area to volume. Based on the above considerations, these parameters will significantly influence the volatilisation rate of free HCN dissolved in water.

On the basis of the physico-chemical properties of HCN (Table 1) and general environmental calculations (US-EPA, 2000), it has been estimated that a cyanide solution of 50 mg CN^-/l evaporates 0.1 mg HCN/cm^2/s. If a pond has a diameter of 100 m, the average mixing height of the air at the edge of the pond downwind is about 6.6 metres. With a wind speed of 1 m/s, the concentration at the edge of the pond is estimated to be approximately $0.1 \times 100/6.6 = 1.5$ mg HCN/m^3. This concentration is expected to decrease rapidly as one moves away from the pond edge due to dispersion in air.

Alternatively, the EQC level III model (Mackay *et al*, 1996) was used to evaluate the evaporation of HCN from water into the air, assuming release of HCN to water only. At steady-state conditions, an amount of 233 kg HCN/h would be released to air from the EQC model water surface (10^{10} m^2) at a concentration of 1.3 mg HCN/m^3. This is equivalent to an evaporation rate of 0.25 mg/m^2/s at a concentration of 50 mg free HCN/l water. If a pond has a diameter of 100 m, the average mixing height of the air at the edge downwind is about 6.6 m. With a wind speed of 1 m/s, the concentration at the edge of the pond is about $0.25 \times 100/6.6 = 3.8$ mg/m^3. At a larger distance, dispersion in air occurs and the concentrations decrease rapidly.

The volatilisation of HCN from cyanide-containing mine wastes was investigated on a laboratory scale using tailings obtained from leaching ores excavated from an oxide, a transition and a sulphide zone. The tests were performed in air-tight glass vessels (2, 5 and 6 l). All tests were performed under the same conditions and on the same mixture (‘pulp’) of oxide, transition and sulphide ores (40% ore and 60% water by weight). The pH was kept constant at 10.5 by addition of sodium hydroxide (NaOH). After 24 hours of leaching, the pH was set to pond conditions (pH 8 - 9) and air (20 l/h) was bubbled through the pulp and then through sodium hydroxide solution (1 mol/l). Samples of the sodium hydroxide solution and 24-hour pulp leach were filtered and analysed for HCN, total CN^-, WAD CN^-, free CN^-, SCN^- and OCN^- using standard methods of analysis (Section 2.5). Approximately 90% of the free cyanide had volatilised as HCN in the case of the oxide ore, and 30% in the case of the sulphide ore. Even when free cyanide could no

longer be measured (below the detection limit of 0.2 ppm), HCN volatilisation was still observed, albeit at a much lower rate due to the stability of the cyanide complexes. (The observation underlines the fact that heavy metal cyanide complexes also form HCN from free cyanide in equilibrium.) The time course of the removal was as follows: free cyanide up to 9 weeks, WAD cyanide up to 6 months, total cyanide approximately 1 year. Depending on the pH, the cyanide concentration, the surface area of the pond and various other parameters, HCN volatilised from the surface continuously, but at a low concentration of typically less than 1 ppm (detection limit of standard HCN detectors) (Rubo *et al*, 2000).

Complexation with transition metals

It has been reported that 28 elements with over 68 oxidation states are capable of forming cyanide-metal complexes (Broderius, 1973). The general formula can be described as $[M^{n+}(CN^-)_x]^{(x-n)-}$ (M = metal ion; the complex can have a positive, negative or neutral charge. Mixed complexes are also common, particularly of the type $[M^{n+}(CN^-)_5X]^{(5-n)-}$ with ligand X = H_2O, NH_3, CO, NO, H or halogen. The complexation reactions involve a number of steps with different equilibrium kinetics that are, however, not well understood at present (Leduc *et al*, 1982; Murgatroyd *et al*, 1998). Dependent on the stability of the various complexes, the metals and/or ligands may be exchanged in the cyanide complex, either by reversible rapid dissociation of the first complex and complexation with another metal, or a substitution process like, for example:

$$[M(CN)_x]^{n-} + H_2O \rightleftarrows [M(CN)_{x-1}H_2O]^{(n+1)-} + CN^- \quad \text{..... (Eq. 13)}$$

The exchange rate is dependent on the total cyanide concentration, pH, temperature and light intensity (Sharpe, 1976 cited by Murgatroyd *et al*, 1998). Copper and nickel cyanide complexes are strongly pH dependent. Increased dissociation and exchange reactions under the influence of light are important for the ferro- (Fe^{2+}) and ferri- (Fe^{3+}) cyanide complexes, $Fe(CN)_6^{4-}$ and $Fe(CN)_6^{3-}$ (Broderius, 1973). Zinc and cadmium cyanide complexes dissociate rapidly, while Ni^{2+}, Cu^{2+} and silver complexes are more stable, but show rapid exchange of the cyanide ligand. Dissociation constants of the most common cyanide complexes are given in Table 9.

Table 9: Dissociation constants of metal-cyanide complexes (Murgatroyd *et al*, 1998)

Species	Dissociation constant [a]	Reference [b]
$Au(CN)_2^-$	38.3	Dean, 1973
$Cd(CN)_4^{2-}$	16.8	Smith, 1976
$Zn(CN)_4^{2-}$	16.7	Smith, 1976
$Ag(CN)_2^-$	–18.7	Broderius, 1973
$Cu(CN)_2^-$	–23.9	Sharpe, 1976
$Cu(CN)_4^{3-}$	–30.7	Sharpe, 1976
$Ni(CN)_4^{2-}$	–31	Broderius, 1973
$Hg(CN)_2$	–32.75	Sharpe, 1976
$Fe(CN)_6^{4-}$	–47	Broderius, 1973
$Fe(CN)_6^{3-}$	–52	Broderius, 1973

[a] Cumulative equilibrium constant, the equilibrium constant for the complete dissociation of the complex ion, irrespective of the intermediate steps

[b] As cited

Abiotic degradation reactions

In principle, HCN can undergo three kinds of abiotic degradation reaction: oxidation, reaction with sulphur and hydrolysis. Oxidation, to carbon dioxide and ammonia via cyanate, can only occur in the presence of strong oxidising agents and is unlikely to occur spontaneously under environmental conditions (Towill *et al*, 1978).

$$2\ HCN + O_2 \longrightarrow 2\ HCNO \xrightarrow{+\ H_2O} 2\ CO_2 + 2\ NH_3 \quad \text{..... (Eq. 14)}$$

Sulphuration to thiocyanate can occur in the presence of reduced sulphur (polysulphide).

$$HCN + S \longrightarrow HSCN \quad \text{..... (Eq. 15)}$$

Hydrolysis, to formic acid and ammonia, is normally a very slow reaction and it does not play a role under environmental conditions. The reaction is accelerated by strongly acidic or alkaline conditions (Kreible and Peiker, 1933; Wiegand and Tremelling, 1972; both cited by Callahan *et al*, 1979).

$$HCN + 2\ H_2O \longrightarrow HCOOH + NH_3 \quad \text{..... (Eq. 16)}$$

During tests run with NaCN (150 µg CN^-/l), potassium ferricyanide (K_3FeCN_3; 185 µg CN^-/l) and cuprous cyanide (CuCN; 180 µg CN^-/l) in sterilised river water at pH 5, 6, 7 or 8, in the light and in the dark, the degradation rate decreased with increasing pH and temperature. At 0, 10, 20 and 30°C, average hydrolysis half-lives were about 23, 15, 10 and 6.8 days, respectively (Chester Engineers, 1977 cited by Fiksel *et al*, 1981).

Photolysis

Under normal environmental conditions, photolysis of HCN is unlikely to occur. In the presence of a catalyst such as titanium dioxide powder, cyanide can be photolytically oxidised to cyanate (Frank and Bard, 1977 cited by Callahan *et al*, 1979).

Photolysis of iron cyanide complexes may be important for the aquatic fate of cyanide, depending on the penetration of UV and visible light (Sharpe, 1976; Broderius and Smith, 1980; both cited by Murgatroyd *et al*, 1998). The extent of photolysis is dependent on the intensity of the light irradiation and the duration of light exposure, which in turn depend on factors such as depth of the water and turbidity, time of the day and latitude.

Photodecomposition of hexacyanoferrate complexes produced 5 moles of CN^- and one mole of cyanate from one mole of hexacyanoferrate (Broderius and Smith, 1980 cited by Murgatroyd *et al*, 1998), while according to Towill *et al* (1978) the reaction can be described by the following equations:

$$4\ Fe(CN)_6^{4-} + O_2 + 2\ H_2O \longrightarrow 4\ Fe(CN)_6^{3-} + 4\ OH^- \quad \text{..... (Eq. 17)}$$

$$4\ Fe(CN)_6^{3-} + 12\ H_2O \xrightarrow{hv} 4\ Fe(OH)_3 + 12\ HCN + 12\ CN^- \quad \text{..... (Eq. 18)}$$

Where *hv* denotes light energy in the form of photons (*h* is Planck's constant, *v* is frequency of light wave)

In distilled water under natural light, the half-life of ferrocyanide was 20 minutes at concentrations between 0.1 to 1 mg $Fe(CN)_6^{4-}$/l. In natural waters, the rate of photodecomposition was 10 times less at a depth of 15 cm and 30 times less at 50 cm compared to the surface (Broderius and Smith, 1980 cited by Murgatroyd *et al*, 1998).

Adsorption

Cyanides are adsorbed by a variety of materials, including clays, biological solids, activated carbon and sediments. In comparison to many refractory organic pollutants, HCN is not strongly partitioned into the sediments or suspended adsorbents, primarily due to its high solubility in water (Callahan *et al*, 1979).

Cyanides are relatively mobile in the soil environment (Alesii and Fuller, 1976), indicating that adsorption is unlikely to be significant in most aquatic environments (cf. Section 4.3.3).

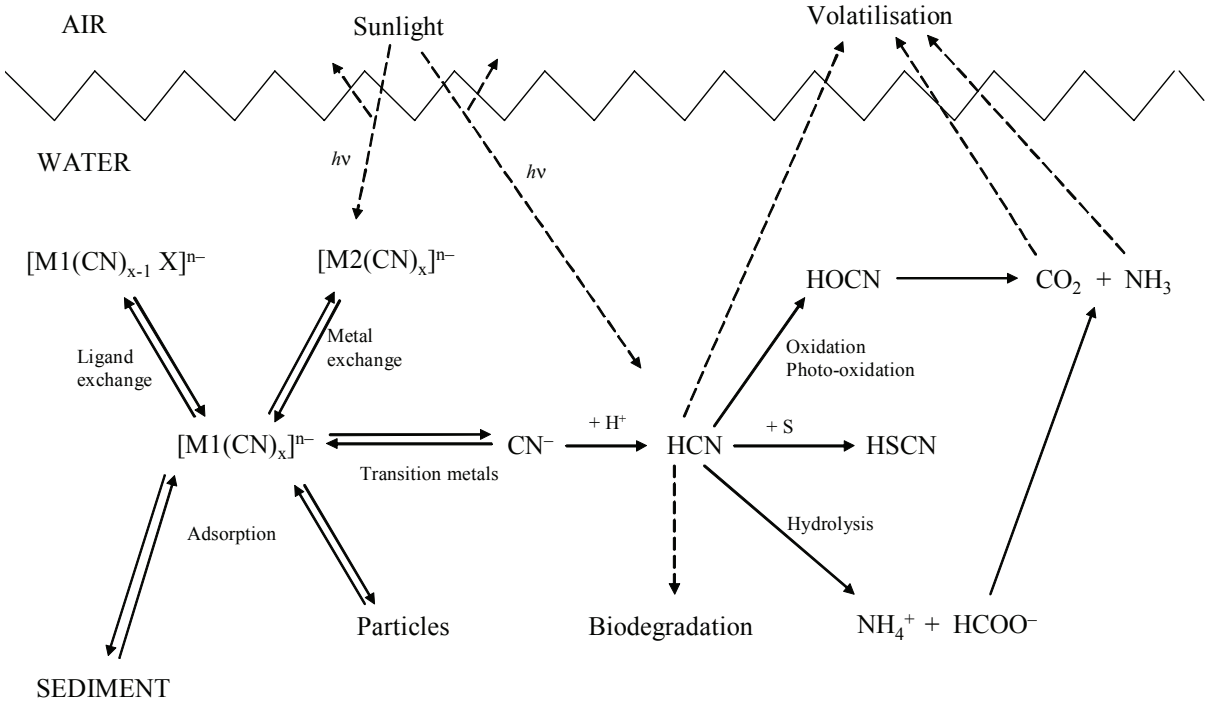
Evaluation of aquatic fate

Cyanide can exist in numerous forms, essentially as free cyanide (which occurs largely in the form of HCN at the pH and temperature range of most water bodies), other less soluble cyanide salts and cyanide complexes. Their fate and behaviour depends largely on the dissociation to free cyanide or adsorption to sediment. Photolysis of cyanide complexes can also liberate free cyanide under the influence of sunlight. To a minor extent cyanides may be oxidised to cyanates, which, in turn, hydrolyse to ammonia and carbon dioxide. Alternatively, in the presence of sulphur, they may be converted into thiocyanates, which are relatively stable in the environment. Direct hydrolysis does not normally play a role.

Volatilisation is expected to be an important, potentially dominant, elimination process for HCN in water. Biodegradation, complexation with subsequent adsorption to sediment and, to a lesser extent, photolysis and abiotic degradation are also possible elimination routes. The rate of removal and persistence of cyanide is governed by the species of cyanide present and the local conditions in the water body. The half-life of free cyanide is mainly governed by volatilisation. It can be in the range of a few days to weeks depending on the wind speed and altitude, the temperature, the surface area, and the surface to volume ratio of the water body. The half-life of complex cyanides may be much longer.

Depending on the environmental conditions, the fate of cyanide in natural waters can be very complex. The most important processes are outlined in Figure 10.

Figure 10: Aquatic fate of HCN[a] (adapted from Murgatroyd *et al*, 1998)



[a] M1 and M2, transition metals; *hv* denotes light energy in the form of photons (*h* is Planck's constant, *v* is frequency of light wave)

4.3.3 Terrestrial fate

As in water, the fate of cyanides in soil is dependent on a number of chemical and biological processes such as redox conditions, pH, complexation or microbial degradation. A comprehensive review of those processes has been published by Kjeldsen (1999).

Cyanides are relatively mobile in the soil environment (Alesii and Fuller, 1976). These authors reported that cyanide mobility is least where soils exhibit low pH, high concentrations of free iron oxides, and positively charged particles (e.g. kaolin, chlorite, gibbsite). Mobility is greatest at high pH, high concentrations of free $CaCO_3$ (high negative charge) and low clay content.

Cruz *et al* (1974 cited by Callahan *et al*, 1979) studied the adsorption of HCN by montmorillonitic clays. The data showed that adsorption is fairly weak and is decreased by the presence of water. Thus, adsorption to montmorillonitic clays is probably not an important fate process in the aquatic environment (Callahan *et al*, 1979) or in soil.

Free cyanide did not adsorb to goethite at different pHs (4 - 10), while potassium ferricyanide, $K_3[Fe(CN)_6]$, adsorbed for 90% at pH 4.0 and 50% at pH 5 (Theis and West, 1986 cited by Murgatroyd *et al*, 1998).

Cyanide carbon and nitrogen were converted to carbonate and ammonia, respectively, in the presence of non-sterile soils. The soils were obtained from areas with and without plants known to contain cyanogenic glucosides. Experiments with doubly labelled potassium cyanide ($K^{14}CN$, $KC^{15}N$) showed that the retention of cyanide nitrogen by the soil is greater than that of cyanide carbon. In general, the soils most actively able to metabolise cyanide (on a specific activity basis) were those sampled from areas supporting plants containing cyanogenic glucosides. Attempts to increase the cyanide metabolising ability of a control soil by pretreatment with 1 mmol KCN/l were unsuccessful, probably because this concentration was inhibitory even to cyanide-metabolising micro-organisms. It was concluded that a cyanide cycle exists in nature, particularly in soil, and that a variety of soils are capable of cyanide metabolism (Strobel, 1967).

Aronstein *et al* (1994) investigated the adsorption of KCN and ferrocyanide, $K_4[Fe(CN)_6]$, to uncontaminated topsoil (pH 6 - 7) and observed that an equilibrium was obtained after 22 days for both forms of cyanide. During the equilibration period, the difference in the amount of free cyanide in the liquid phase between the soil treated with KCN and ferrocyanide decreased from 23% to 11%. At equilibrium, the free cyanide concentration in the liquid was higher with ferrocyanide than with KCN. The absolute adsorption was 80.8% in the for KCN and 94% for ferrocyanide. Desorption experiments through weathering revealed that the maximum amount released from topsoil decreased from 10% at day 10 to 1.5% at day 60 for the samples treated with KCN and was 4.3% at day 35 for ferrocyanide. Later, desorption increased again, but did not reach the initial figures. The time necessary to reach the maximum extent of adsorption increased from 20 hours at day 2 to 144 hours (6 d) at day 60 for both forms indicating a decreasing availability with time. The authors explained the small differences in adsorption between ‘free cyanide’ (KCN) and ‘complex’ ferrocyanide by an initial reaction of cyanide with metal ions contained in the soil to form complexes. The secondary adsorption of those complexes to the soil surface was similar to the adsorption of the hexacyanoferrate complexes when applied directly. The decrease in desorption with time was explained by a redistribution of adsorbed cyanide between external and internal sorption sites of the soil.

The same authors conducted a study to determine the effect of various factors on the rate and extent of KCN and potassium ferrocyanide complex, $K_4Fe(CN)_6$, removal from aqueous soil-containing systems using ^{14}C-labelled cyanide solutions with a concentration of 25 mg/l HCN. In a sterile aqueous system at neutral pH, the concentration of free cyanide was reduced by 42% in 334 hours (13.9 d) as a result of the protonation of CN^- and the volatilisation of the HCN formed. In the presence of an aerobic mixed consortium of micro-organisms isolated from cyanide-contaminated soil from a manufactured gas plant, a cyanogenic, methylotrophic culture

(*Pseudomonas* species, "Isolate 3"), the concentration of free cyanide was reduced by 59% and 66% in 357 hours (14.9 d), respectively. This resulted from combined chemical conversion and microbial degradation. Most (90%) of the cyanide removed was detected as CO_2. In the sterile aqueous system amended initially by the complex form of cyanide, less than 20% reduction in cyanide occurred. The adsorption equilibrium for free and complex cyanides in slurries of the topsoil and soil from a manufactured gas plant was reached in less than 22 and 4 days, respectively (Aronstein *et al*, 1994). It should be noted, however, that these experiments, even though performed with soil slurries and soil micro-organisms, took place under aqueous conditions.

Zintgraff *et al* (1969) found respiratory inhibition of soil microflora after exposure to cyanide at concentrations similar to those which caused effects in activated sludge (0.26 mg CN^-/l). Cyanide also inhibited nitrification.

Cyanide ions are not strongly adsorbed or retained by soils (Murrmann and Koutz, 1972). The cyanide salts of most cations are soluble (except AgCN) but move only a short distance through soil before being biologically converted under aerobic conditions to nitrates (by microbial degradation to NH_3, then conversion to NO_3^-) or fixed by trace metals through complex formation. Under anaerobic conditions, cyanides denitrify to gaseous nitrogen compounds which enter the atmosphere. The cyanide ion is not involved in oxidation-reduction reactions (Murrmann and Koutz, 1972).

The presence of roots of trees and other plants may also influence the fate of cyanide in soil. Experimental work with cyanide solution and contaminated soil from an old city gas factory demonstrated the ability of white willows (*Salix alba*) to remove cyanide. At contaminated sites cyanide was mainly present in the form of iron complexes. Mass balance experiments showed that cyanide was metabolised because the amount absorbed by the roots was higher than the added amounts present in plant tissues and volatilised by leaves. The same ability to remove cyanide was found in other tree species (Larsen *et al*, 2004). Other plants have also been investigated for their suitability in phyto-remediation of cyanide-contaminated sites (Yu *et al*, 2004).

Conclusion

The fate of cyanide in soil is the result of a complex interaction of different factors. These factors may be physical, chemical and physico-chemical (e.g. pH, volatilisation, water content, content of sulphur compounds, presence of complexing agents/equilibrium between free and complex cyanide, absorption) and biological or biochemical (e.g. presence of organisms with the capacity to metabolise cyanide, toxicity). Whether volatilisation, adsorption, complexation or

biodegradation play the major role in a particular soil environment depends on the presence or absence of those factors which influence the behaviour of cyanide.

Several aspects regarding the biodegradation and metabolism of cyanide by soil-dwelling micro-organisms and higher plants are described in the next section.

4.3.4 Metabolism

Cyanide may be biodegraded or metabolised by all life-forms using a wide variety of metabolic pathways, described in this section. In vertebrates, the major pathway is the formation of thiocyanate by the enzyme rhodanese (Section 7.3). This pathway plays only a minor role in the environment as a whole. Important pathways of cyanide metabolism in the environment are described in this section. This general overview is followed by a review of specific biodegradation studies (Section 4.3.5).

Cyanide metabolism includes biological assimilation, detoxification, degradation, removal (elimination), and use as nutrition/energy source. In principle, living organisms utilise five different enzymatic pathways for the metabolism of cyanide, based on the underlying reactions: (i) substitution (e.g. addition and formation of β-cyanoalanine, an amino acid precursor), (ii) hydrolysis (yielding formate), (iii) oxidation, (iv) transfer (of sulphur: thiocyanate formation) and (v) (anaerobic) reduction. These pathways are available in various organisms, ranging from bacteria (Castric, 1981) and fungi to plants and mammals. Almost all the corresponding enzymes have been identified and given EC numbers [a]. Their reaction kinetics have been elucidated, and the responsible genes sequenced in several organisms (described elsewhere, beyond the scope of this review).

Several pathways start with destruction of the –C≡N triple bond. Typical metabolites are formamide, formate and ultimately carbon dioxide and ammonia. Oxidation and (anaerobic) reduction are the only pathways leading to complete mineralisation of cyanide (into ammonia and carbon dioxide or methane, respectively). The hydrolysis pathway stops at formate. The other two pathways – substitution (β-cyanoalanine), and transfer (thiocyanate) – are fundamentally different in that they are based on an expendable substrate (amino acid precursor or sulphur donor), while the capacity of the hydrolysis (formate) pathway is only limited by kinetic parameters.

Cyanide assimilation (N-fixation) via β-cyanoalanine seems to be the major metabolic pathway in the environment, particularly in plants, but also in fungi and bacteria. It is an alternative to molecular nitrogen (N_2) fixation and synthesis of certain amino acids, e.g. asparagines and

[a] For example, rhodanase is classified as EC 2.8.1.1 - thiosulphate:cyanide sulphur transferase (IUBMB, 1999)

aspartases. Other, more important pathways exist to synthesise these amino acids. Rhodanese, the major enzyme system in vertebrates, plays – though present in many cases – only a minor role in the environment, probably because sulphur is too valuable to be used for the purpose of cyanide fixation/detoxification.

All reactions have been more or less successfully exploited as industrial processes for the biodegradation and bioremediation of cyanide-containing wastewater or sediment/soil contaminated with cyanide. For example, hydrolysis and oxidation and, more recently, reduction processes are used for effluent treatment (Section 4.3.5). Assimilation and thiocyanate formation are also applied, e.g. in soil- and/or phyto-remediation. In wastewater, cyanide solutions containing up to 100 mg CN^-/l can normally be treated, in spite of the known inhibitory effect of cyanide on respiration (by binding to cytochrome *c* oxidase and other metallo-proteins). The toxicity can partially be overcome by gradual acclimatisation to progresssively higher cyanide concentrations (Knowles, 1976; Raybuck, 1992; Ebbs, 2004). The toxicity of cyanide to micro-organisms and activated sludge is reviewed in Section 6.1.

Some organisms are capable of using more than one pathway, probably influenced by environmental factors such as oxygen, pH and cyanide concentration, and the presence of interfering (inhibitory) compounds. In soil and water, bio-availability and solubility may also play a role. For example, cultures of *Chromobacterium violaceum* grown on glutamate with or without addition of glycine, produced less or more HCN, respectively, during the idiophase (Section 4.1.3 *Bacteria*). The low cyanide concentrations in turn induced several cyanide detoxifying enzymes that enabled formation of β-cyanoalanine (and then aspartate that is further metabolised), γ-cyano-α-aminobutyric acid (by-product without further metabolism), and thiocyanate (Rodgers and Knowles, 1978).

Organisms known to metabolise free cyanide (e.g. bacterium: *Pseudomonas fluorescens*, fungus: *Fusarium solani* and higher plant: willow *Salix eriocephala*) can often also degrade cyanates (especially thiocyanates), nitriles, and Fe- and Ni-complexes of cyanide. Degradation of nitriles leads to formation of cyanide (Section 4.1.3), which can then be further degraded and/or cause autolysis (if formation exceeds breakdown) of the competing and/or the producing organism itself.

The following text summarises the five different metabolic pathways following earlier reviews by Knowles (1976), Castric (1981) and Raybuck (1992) and a recent mini-review by Ebbs (2004), where further specific details, reviews and references can be found. These reviews focus on bacterial and fungal degradation (with a view to potential biodegradation and bioremediation). Duffey (1981) and Fry and Myers (1981) briefly reviewed the metabolism of cyanogenic arthropods (insects) and pathogenic fungi, respectively. No review was available on metabolism in other organisms, particularly higher plants.

For cyanide metabolism in mammals, the reader is referred to Section 7.3. Examples of various organisms in which the different pathways haven been found are tabulated underneath the five pathways discussed below (Table 10).

1. Substitution

   β-Cyanoalanine formation is a substitution reaction which involves 'condensation' (addition) of HCN with cysteine or serine into β-cyanoalanine, catalysed by cyanoalanine synthase, a pyridoxal phosphate-dependent enzyme found in bacteria, algae, insects and higher plants. The cyanide carbon is incorporated only in the C-3 position of β-cyanoalanine, while the –C≡N triple bond is retained in the cyano group of this amino acid precursor (even in cyanogenic *C. violaceum* which assimilates the HCN formed from glycine). The reaction liberates hydrogen sulphide.

$$\text{Cysteine} + CN^- \xrightarrow{\textit{Cyanoalanine synthase}} \beta\text{-Cyanoalanine} + H_2S \quad \text{(Eq. 19)}$$

   In blue lupin (*Lupinus angustifolia*) and potato tubers (*Solanum tuberosum*), β-cyanoalanine synthase condenses HCN and cysteine, but also – though less efficiently – generates cysteine from O-acetyl-L-serine and hydrogen sulphide. In addition, another enzyme, cysteine synthase that normally generates L-cysteine from hydrogen sulphide and O-acetyl-L-serine, can convert HCN and O-acetyl-L-serine to β-cyanoalanine and acetate, as a side reaction at a low rate. The key difference seems to be the selectivity of the active site for the incoming HCN or hydrogen sulphide.

$$\text{O-Acetyl-L-serine} + CN^- \xrightarrow{\textit{Cysteine synthase}} \beta\text{-Cyanoalanine} + CH_3COO^- \quad \text{(Eq. 20)}$$

   In both cases, the β-cyanoalanine thus formed can be further hydrolysed by asparaginases, in bacteria and insects, to L-asparagine and aspartic acid (releasing ammonia) or, in plants predominantly to L-asparagine, and/or also γ-glutamyl-β-cyanoalanine, e.g. in vetch plants (*Vicia* sp.) (Fowden and Bell, 1965). Blue lupin (*L. angustifolia*), possesses β-cyanoalanine nitrilase that will catalyse the first step, but not further hydrolyse L-asparagine. In the cyanogenic bacterium *C. violaceum*, both enzymes are present (cysteine synthase 84% active compared with true synthase), while β-cyanoalanine is the major end product; however, no

cyanide appears to be incorporated into asparagine or aspartic acid (Figure 11 below, upper part).

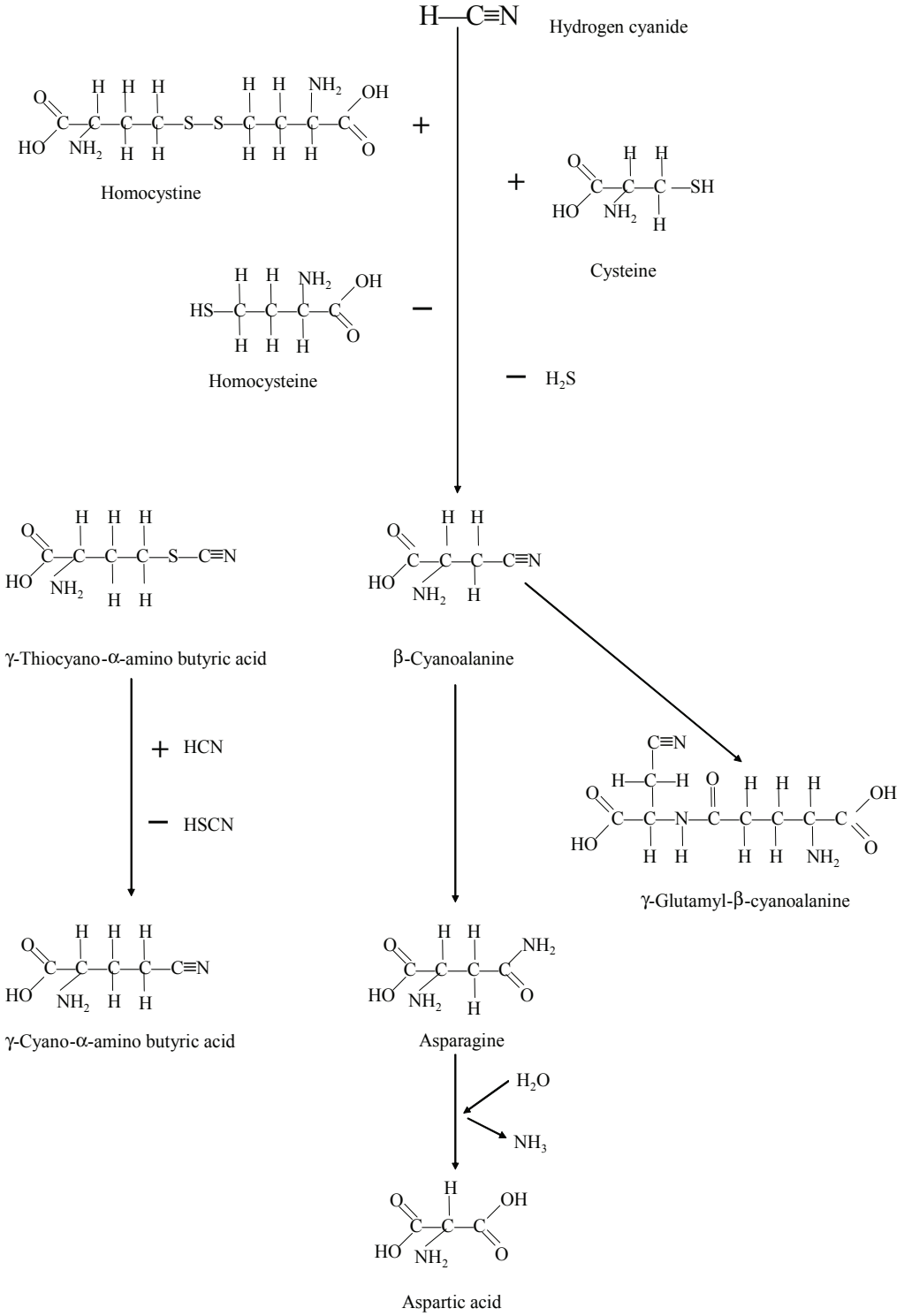
An exceptional substitution reaction is the synthesis of γ-cyano-α-aminobutyric acid in *C. violaceum*. The overall reaction involves two steps, starting with non-enzymatic formation of γ-thiocyano-α-aminobutyric acid (S-cyano homocysteine) and homocysteine, from homocystine. A specific synthase then catalyses the condensation of HCN to yield thiocyanate (as above) and γ-cyano-α-aminobutyric acid.

$$CN^- + \text{homocystine} \longrightarrow \gamma\text{-thiocyano-}\alpha\text{-aminobutyric acid} + \text{homocysteine} \quad \text{(Eq. 21)}$$

$$CN^- + \gamma\text{-thiocyano-}\alpha\text{-aminobutyric} \xrightarrow{\textit{Synthase}} SCN^- + \gamma\text{-cyano-}\alpha\text{-aminobutyric acid} \quad \text{(Eq. 22)}$$

The synthase requires pyridoxal phosphate as a co-factor, and performs optimally at pH 8.3 to 10.5. Thiocyanate is an inhibitor at increasing concentrations, but not γ-cyano-α-aminobutyric acid. The physiological role of this reaction sequence is unclear, but it may be regarded as a detoxification mechanism since the synthase level increases during the idiophase of cyanogenesis and γ-cyano-α-aminobutyric acid is not further metabolised (Castric, 1981 and reference therein) (Figure 11 below, lower part).

Figure 11: Cyanide assimilation via β-cyanoalanine (upper part) or γ-cyanoaminobutyric acid (lower part) (adapted from Castric, 1981)



2. Hydrolysis

Hydrolytic reactions are catalysed by hydrolases, the primarily fungal enzyme cyanide hydratase which forms formamide or the mainly bacterial enzyme cyanidase (cyanide dihydratase) which yields formate and ammonia. Cyanidase also occurs in higher animals (Section 7.3, reaction 4). In both cases, the actual substrate is probably HCN, rather than CN^-.

$$HCN + H_2O \xrightarrow{\textit{Hydratase}} HCONH_2 \quad \text{(Eq. 23)}$$

$$HCN + 2\ H_2O \xrightarrow{\textit{Cyanidase}} HCOOH + NH_3 \quad \text{(Eq. 24)}$$

Formamide, formic acid and ammonia can, under certain conditions, be used as sources of nutrition (N) or energy (C) and enhance growth. The enzymatic hydrolysis of nitriles has an analogous role. The structure and amino acids of hydratase and cyanidase show their similarity to nitrile hydratases found in many bacteria, fungi, yeasts and plants.

Both reactions destroy the –C≡N triple bond completely, eliminating the possibility of further reactivity. They yield less toxic products, thus enabling an effective detoxification mechanism (e.g. used by fungi upon invasion of a cyanogenic plant like sorghum). Enzymatic hydrolysis does not require cofactors and also occurs in the absence of viable cells, as long as the functional enzymes are present. Hydrolysis can also occur abiotically (Section 4.3.2) or non-enzymatically in the Kiliani reaction (Pathway 5, reduction).

3. Oxidation

Oxidative reactions form ammonia and carbon dioxide, in two steps: formation of cyanate catalysed by the cyanide mono-oxygenase and subsequent conversion of cyanate, catalysed by cyanase, into ammonia and carbon dioxide. Cyanases are present in many living organisms, in bacteria, plants (as ammonia assimilator) and as detoxification mechanism in mammals (Section 7.3, reaction 3). In *Escherichia coli* and *Pseudomonas fluorescens*, prior cyanohydrin complexation was necessary to allow oxygenase-mediation, but this may not be an absolute requirement in all species.

$$HCN + O_2 + H+ + NAD(P)H \xrightarrow{\textit{Mono-oxygenase}} HCON + NAD(P)+ + H_2O \quad \text{(Eq. 25)}$$

$$[H^+]\ [O–C\equiv N^-] + H_2O \xrightarrow{\textit{Cyanase}} NH_3 + CO_2 \quad \text{(Eq. 26)}$$

Another oxidative pathway involves cyanide dioxygenase to form ammonia and carbon dioxide directly, probably requiring a pterin cofactor (a pterin is composed of a pyrazine ring and a pyrimidine ring; the pyrimidine ring having a carbonyl oxygen and an amino group).

$$HCN + O_2 + H^+ + NAD(P)H \xrightarrow{\textit{Dioxygenase}} NH_3 + CO_2 + NAD(P)^+ \quad \text{(Eq. 27)}$$

Where NAD(P)H = reduced nicotinamide adenine dinucleotide (phosphate)

Oxidation may also occur abiotically (Section 4.3.2).

4. Transfer

Thiocyanate formation is a transfer reaction, involving trans-sulphuration of cyanide with thiosulphite into thiocyanate. It is catalysed by rhodanese occurring in a variety of bacteria, plants, fungi, insects and vertebrates, including fish and mammals (Section 7.3, reaction 1). The reaction liberates sulphite.

$$CN^- + S_2O_3^{2-} \xrightarrow{\textit{Rhodanese}} SCN^- + SO_3^{2-} \quad \text{(Eq. 28)}$$

Rhodanese catalysis occurs in two steps, via an enzyme-persulphide or -thiosulphate intermediate. High concentrations of rhodanese are found in mammalian liver mitochondria.

Another enzyme responsible for thiocyanate formation is 3-mercaptopyruvate sulphotransferase [a], demonstrated in bacteria and in mammals (Section 7.3, reaction 1). The enzyme requires both substrates to be bound in a ternary complex before direct sulphur transfer occurs (contrary to two-steps needed by rhodanese).

$$CN^- + \text{mercaptopyruvic acid} \xrightarrow{\textit{Sulphotransferase}} SCN^- + \text{pyruvic acid} \quad \text{(Eq. 29)}$$

The thiocyanate formed by either route is less toxic and normally excreted, and the reaction may thus be regarded as a significant HCN detoxification mechanism. In mammals it is the major detoxification route (Section 7.3, reaction 1). However, the primary role of both

[a] EC 2.8.1.2: 3-mercaptopyruvate:cyanide sulphurtransferase, synonym β-mercaptopyruvate sulphotransferase (IUBMB, 1999)

sulphur transferase enzymes is in the metabolism of sulphane sulphur (labile –SH) and iron sulphur synthesis.

The excreted thiocyanate can be utilised as nitrogen and carbon (energy) sources by other bacteria such as *Thiobacillus*, *Arthrobacter* and several *Pseudomonas* species. Alternatively, thiocyanate is further utilised by the producing organism itself, allowing chemolithotrophy as seen in certain bacteria (salt lake *Thiobacillus thioparus*), the fungus *Acremonium strictum*, and recently *Klebsiella oxytoca*. These organisms are able to convert thiocyanate into ammonia and carbonyl sulphide (hydrolase), or ammonia and carbon dioxide via cyanate (cyanase).

5. Reduction

Reduction of cyanide occurs under anaerobic, or mixed anaerobic/aerobic, biodegradation conditions. HCN may serve as an alternate substrate for nitrogenase, which is capable of reducing dinitrogen (N_2). The enzyme consists of two metalloproteins (MoFe and Fe) and aids in the nitrogen fixation by symbiotic plant bacteria such as symbiotic *Rhizobium*. Since CN^- is an inhibitor blocking electron flow, the reaction favours acidic conditions, and HCN is the preferred substrate. The postulated intermediate transformation product CH_2=NH is hydrolysed into formaldehyde, and further reduced into methane and ammonia.

$$HCN + 2H^+ + 2e^- \xrightarrow{\textit{Nitrogenase}} [CH_2{=}NH] \xrightarrow{+\,H_2O} CH_2{=}O \quad \text{..... (Eq. 30)}$$

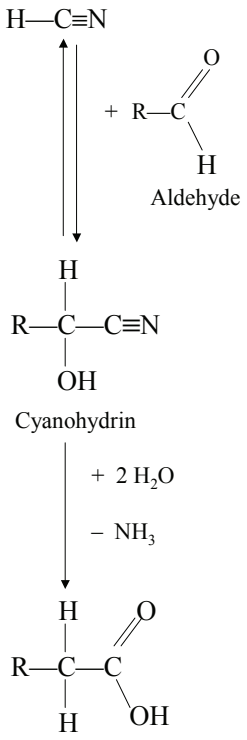
$$[CH_2{=}NH] + 2H^+ + 2e^- \xrightarrow{\textit{Nitrogenase}} CH_3{-}NH_2 + 2H^+ + 2e^- \longrightarrow CH_4 + NH_3 \quad \text{..... (Eq. 31)}$$

In a study of cassava processing wastes (containing cyanides), methanogenesis was solely due to the genus *Methanothrix* (Paixao *et al*, 2000, below). Otherwise, methanogens are notorious for their sensitivity to cyanide. After an over-dose of cyanide, methanogenesis resumes only after a long lag-time. The formation of the main end product, ammonia, could also be due to abiotic hydrolysis of HCN (Section 4.3.2 and Kiliani reaction below). For example, the Kiliani conversion could explain the considerable lag time needed for acclimation of *Klebsiella planticola* grown on cyanide (5 mmol/l) and glucose (70 mmol/l). After depletion of cyanide by the Kiliani reaction, microbial growth commenced on the remaining sugar and newly formed ammonia (Hope and Knowles, 1991).

The bacterium *Klebsiella oxytoca* strains can degrade cyanide by both reductive (resting cells) and oxidative pathways (following cyanide treatment).

Under anaerobic conditions, certain sugars may cause non-enzymatic conversion (hydrolysis) of cyanide to ammonia. This so-called Kiliani reaction is well-documented for reducing sugars such as galactose, mannose, fructose and ribose. It involves initial cyanohydrin formation between the carbonyl group of the reducing sugar and cyanide ion. Hydrolysis of the cyanohydrin is accelerated by an intra-molecular hydroxyl group to give ammonia. The sugar now possesses a carboxylic acid group and has been extended by one carbon atom (Figure 12).

Figure 12: Kiliani reaction



Examples of various organisms in which the different pathways haven been found are listed in Table 10 below. Individual data were taken from references cited by the above reviews (Knowles, 1976; Duffey, 1981; Raybuck, 1992; Ebbs, 2004) and/or from original literature sources. As stated above, the amount and level of specific activity of the relevant enzymes, and the importance of the respective metabolic pathway, may differ considerably. Furthermore, the table is skewed towards microbial organisms, with a view to their use in biodegradation and bioremediation.

Table 10: Metabolic pathways and products in different organisms

Pathway: initial product/ Organism: order	Species	Remark	Reference, primary and/or (secondary)
1a. Substitution: β-cyanoalanine			
Bacteria	*Escherichia coli*		(Dunnill and Fowden, 1965 cited by Castric, 1981) (Akopyan *et al*, 1975 cited by Raybuck, 1992)
	Bacillus megaturium		(Castric and Strobel, 1969; Castric and Conn, 1971 both cited by Castric, 1981) (Akopyan *et al*, 1975 cited by Raybuck, 1992)
	Pseudomonas sp.		(Yanase *et al*, 1983 cited by Raybuck, 1992)
	Chromobacterium violaceum		Rodgers and Knowles, 1978; Rodgers, 1981 (Brysk *et al*, 1969a,b both cited by Castric, 1981) (Akopyan *et al*, 1975; Macadam and Knowles, 1984 both cited by Raybuck, 1992)
Fungi	*Pholiota* sp., *Marasmus oreades*, *Fusarium nivale*, *Rhizopus nigricans*	Preferably under anaerobic conditions	Allen and Strobel, 1966
	Snow mould basidiomycete		(Strobel, 1966 cited by Chamberlain and MacKenzie, 1981)
Algae	*Chlorella pyrenoidosa*		Fowden and Bell, 1965
Plants	*Lupinus angustifolia* (blue lupin)	(Substitution of SH in cystein is preferred to substitution of OH in serin. The latter occurs, but to a minor extent (all plants examined)	Blumenthal *et al*, 1968; Hendrickson and Conn, 1969; Lever and Butler, 1971; Castric *et al*, 1972
	Solanum tuberosum (potato)		Maruyama *et al*, 2000

Table 10: Metabolic pathways and products in different organisms (cont'd)

Pathway: initial product/ Organism: order	**Species**	**Remark**	**Reference, primary and/or (secondary)**
Plants (cont'd)	*Spinacia oleracea* (spinach)		(Hatzfeld *et al*, 2000; Ikegami *et al*, 1988a,b cited by Raybuck, 1992)
	Arabidopsis thaliana		(Hatzfeld *et al*, 2000; Ikegami *et al*, 1988a,b cited by Raybuck, 1992)
	Zea mays (corn)	Roots	Oaks and Johnson, 1972
	Lotus arbicus, *Lotus tenuis*	Etiolated seedlings	Floss *et al*, 1965; Abrol and Conn, 1966; Blumenthal *et al*, 1968
	Sorghum vulgare sp.		Blumenthal-Goldschmidt *et al*, 1963; Blumenthal *et al*, 1968
	Hordeum vulgare sp. (barley)		Blumenthal-Goldschmidt *et al*, 1963
	Pisum sativum sp. (pea)		Blumenthal-Goldschmidt *et al*, 1963
	Linum usitatissimum sp. (flax)		Blumenthal-Goldschmidt *et al*, 1963; Blumenthal *et al*, 1968
	Trifolium pratense sp. (red clover)		Blumenthal-Goldschmidt *et al*, 1963
	T. repens sp. (white clover)		Blumenthal-Goldschmidt *et al*, 1963; Blumenthal *et al*, 1968
	Cucumis sativus (cucumber, gherkin)		Fowden and Bell, 1965
	Ecballium elaterium (squirting cucumber)		Fowden and Bell, 1965
	Lathyrus odoratus (sweet pea)		Fowden and Bell, 1965
	Vicia monantha, *V. ferruginosa*, *V. lutea*, *V. faba* (vetch)		Fowden and Bell, 1965
	V. sativa (vetch)		Fowden and Bell, 1965; Blumenthal *et al*, 1968

Table 10: Metabolic pathways and products in different organisms (cont'd)

Pathway: initial product/ Organism: order	**Species**	**Remark**	**Reference, primary and/or (secondary)**
Arthropoda: Diplopoda	*Oxidus gracilis*, *Harpaphe haydeniana*	Millipedes	(Duffey and Blum, 1977; Duffey and Towers, 1978 both cited by Duffey, 1981)
	Spodoptera eridania (southern army worm)	Moth	Meyers and Ahmad, 1991
Arthropoda: Insecta	*Trichoplusia ni* (cabbage looper)	Moth	Meyers and Ahmad, 1991
	Heliothis zea (corn earworm), *Spodoptera exigua* (beet army worm) larvae	Moths	(Duffey, unpublished data cited by Duffey, 1981)
	Oncopeltus fasciatus (milkweed bug) adults	Heteroptera: Lygaeidae	(Duffey, unpublished data cited by Duffey, 1981)
1b. Substitution: γ-cyanoaminobutyric acid			
Bacteria	*C. violaceum*		Rodgers and Knowles, 1978; Rodgers, 1981 (Brysk and Ressler, 1970; Ressler *et al*, 1973; Akopyan *et al*, 1975 all three cited by Castric, 1981)

Table 10: Metabolic pathways and products in different organisms (cont'd)

Pathway: initial product/ Organism: order	**Species**	**Remark**	**Reference, primary and/or (secondary)**
1c. Substitution: α-aminobutyronitrile and α-aminobutyric acid			
Fungi	*Rhizoctonia solani* (soil-borne pathogenic basidiomycete)	Reaction of CN^- with propionaldehyde to α-amino-butyronitrile and α-aminobutyric acid)	(Mundy *et al*, 1973 cited by Fry and Myers, 1981)
2a. Hydrolysis: Formate and ammonia			
Bacteria	*Pseudomonas* sp.	Acclimated, up to 100 mg CN^-/l + lactate	White *et al*, 1988
	Actinomycetes sp.		(Painter and Ware, 1955 cited by Howe, 1965)
	P. fluorescens	Preferably under anaerobic conditions	Kunz *et al*, 1992
	Alcaligenes xylosoxidans denitrificans	Cyanidase; 100 - 1,000 mmol/l	(Ingvorsen and Godtfredsen, 1988 cited by Raybuck, 1992) Basheer *et al*, 1992
2b. Hydrolysis: Formamide			
Bacteria	*P. fluorescens*	Preferably under anaerobic conditions	Kunz *et al*, 1992
	E. coli gene transformed	Cyanide hydratase gene from *Fusarium solani*	(Nolan *et al*, 2003 cited by Ebbs, 2004)

Table 10: Metabolic pathways and products in different organisms (cont'd)

Pathway: initial product/ Organism: order	**Species**	**Remark**	**Reference, primary and/or (secondary)**
Fungi	*Stemphylium loti*	Spores, after adaptation to cyanide	Fry and Millar, 1972; Fry and Myers, 1981 (Nazly and Knowles, 1981; Nazly *et al*, 1983 both cited by Raybuck, 1992)
	Fusarium solani		(Richardson, 1987; Richardson and Clarke, 1987 both cited by Raybuck, 1992) (Barclay *et al*, 2002 cited by Ebbs, 2004)
	Gloeocercospora sorghi		Fry and Myers, 1981 (Thatcher and Weaver, 1976 cited by Castric, 1981) (Fry and Evans, 1977; Nazly and Knowles, 1983 both cited by Raybuck, 1992)
	Helminthosporium turcicum		(Fry and Evans, 1977 cited by Raybuck, 1992)
	H. maydis		Fry and Myers, 1981
3. Oxidation: Cyanate, ammonia, carbon dioxide			
Bacteria	*E. coli*		(Kunz *et al*, 2001; Figuera *et al*, 1996 both cited by Ebbs, 2004)
	P. fluorescens		Kunz and Nagappan, 1989; Kunz *et al*, 1992 (Kunz *et al*, 1998 cited by Ebbs, 2004) (Harris and Knowles, 1983; Rollinson *et al*, 1987; Dorr and Knowles, 1989; all three cited by Raybuck, 1992)

Table 10: Metabolic pathways and products in different organisms (cont'd)

Pathway: initial product/ Organism: order	**Species**	**Remark**	**Reference, primary and/or (secondary)**
Bacteria (cont'd)	*Pseudomonas* sp., *Vibriones* sp., *Enterobacterium* sp.		Boucabeille *et al*, 1994
	P. putida		Babu *et al*, 1992, 1996 (Silva-Avalos *et al*, 1990 cited by Raybuck, 1992)
	Bacillus pumilus	Soil and dead plants	Skowronski and Strobel, 1969; Meyers *et al*, 1991
	P. paucimobilis	Mining waste	Mudder and Whitlock, 1984
	Flavobacterium sp.		(Unspecified reference cited by Raybuck, 1992)
	Klebsiella oxytoca	Resting cells	(Kao *et al*, 2003 cited by Ebbs, 2004)
Fungi	*Phanerochaete chrysosporium*		Shah *et al*, 1991 (Shah *et al*, 1989 cited by Raybuck, 1992)
	Fusarium solani		Shimizu *et al*, 1969
Plants	*Salix eriocephala* (willow)		Ebbs *et al*, 2003 (Guilloton *et al*, 2002 cited by Ebbs, 2004)
4. Transfer: Thiocyanate			
Bacteria	*E. coli*		Villarejo and Westley, 1963; Volini and Alexander, 1981 (Vachek and Wood, 1972 cited by Castric, 1981 or Raybuck, 1992) (Westley, 1988 cited by Raybuck, 1992) (Jarabak, 1981; Pagani *et al*, 1991 both cited by Raybuck, 1992)

Table 10: Metabolic pathways and products in different organisms (cont'd)

Pathway: initial product/ Organism: order	**Species**	**Remark**	**Reference, primary and/or (secondary)**
Bacteria (cont'd)	*Neurospora crassa*		Villarejo and Westley, 1963
	Bacillus subtilis		Villarejo and Westley, 1963
	B. coagulans		Villarejo and Westley, 1963
	Thiobacillus denitrificans		(Bowen *et al*, 1965 cited by Castric, 1981) (Westley, 1988 cited by Raybuck, 1992)
	Rhodospirillum palustris		(Yoch and Lindstrom, 1971 cited by Castric, 1981)
	Klebsiella pneumoniae		(Westley, 1988 cited by Raybuck, 1992)
	B. stearothermophilus		Villarejo and Westley, 1963; Atkinson, 1975
	R. palustris		(Westley, 1988 cited by Raybuck, 1992)
	Azobacter vinelandii		(Westley, 1988 cited by Raybuck, 1992)
	P. aeruginosa		Volini and Alexander, 1981 (Schook and Berk, 1978; Vandenbergh *et al*, 1979 both cited by Castric, 1981) (Westley, 1988 cited by Raybuck, 1992)
	C. violaceum		Rodgers and Knowles, 1978

Table 10: Metabolic pathways and products in different organisms (cont'd)

Pathway: initial product/ Organism: order	**Species**	**Remark**	**Reference, primary and/or (secondary)**
Arthropoda: Diplopoda	*Oxidus gracilis*, *Harpaphe haydeniana*	Cyanogenic millipedes. SCN may be due to symbiotes in reaction chamber	(Duffey and Blum, 1977; Duffey and Towers, 1978 both cited by Duffey, 1981)
Arthropoda: Insecta	*Zygaena filipendulae*, *Z. lonicerae* (moths), *Polyommatus icarus* (butterfly)	May be due to symbiotes or endoparasites	(Parsons and Rothschild, 1964 cited by Duffey, 1981)
	Heliothis zea, *Spodoptera exigua* (noctuid moths) larvae		(Duffey, unpublished data cited by Duffey, 1981)
	Oncopeltus fasciatus (milkweed bug) adults	Heteroptera: Lygaeidae	(Duffey, unpublished data cited by Duffey, 1981)
Fish	*Oncorhynchus mykiss* (rainbow trout)		(Raymond, 1979 cited by Ruby *et al*, 1986) Lanno and Dixon, 1996
	Cyprinus carpio (carp)		(Sido and Koji, 1972 cited by Ruby *et al*, 1986)
Mammals		Bovine liver, kidney, heart, adrenal gland *in vitro*	Villarejo and Westley, 1963
5. Reduction: methane and ammonia			
Bacteria	*Rhizobium ORS571*		Stam *et al*, 1985
	Methanothrix sp.		Paixao *et al*, 2000
	Klebsiella oxytoca	Cyanide-enriched wastewater	(Kao *et al*, 2003 cited by Ebbs, 2004)

Table 10: Metabolic pathways and products in different organisms (cont'd)

Pathway: initial product/ Organism: order	**Species**	**Remark**	**Reference, primary and/or (secondary)**
Bacteria (cont'd)	*Azotobacter chroococcum*		(Kelly, 1968 cited by Jaeger and Dotterweich, 1986)
	Clostridium pasteurianum		(Hardy and Knight, 1967 cited by Jaeger and Dotterweich, 1986)
	A. vinelandii		(Hardy and Knight, 1967 cited by Jaeger and Dotterweich, 1986)
	Unknown	5 - 100 mg/l	Jaeger and Dotterweich, 1986
	Unknown	> 100 mg/l	Fallon *et al*, 1991

Chromobacterium violaceum is an example of how metabolism of bacterial cyanide and cyanogenesis are intertwined. The methylene nitrogen and amino nitrogen of glycine serve as precursor for cyanide, which in turn serves as a precursor for the cyano group of β-cyanoalanine. Glycine is a one-carbon donor which condenses with another molecule of glycine to form serine. Serine, in turn, is the direct or indirect three carbon precursor of β-cyanoalanine. During the idiophase, where maximum cyanogenesis occurs, synthesis of cyanoalanine synthase, rhodanese and γ-cyano-α-aminobutyrase is stimulated, causing a sharp decrease in HCN levels. In contrast, cyanogenic pseudomonads did not further metabolise their own HCN production, although rhodanese was present (Castric, 1981 and references therein).

Detoxification in arthropods

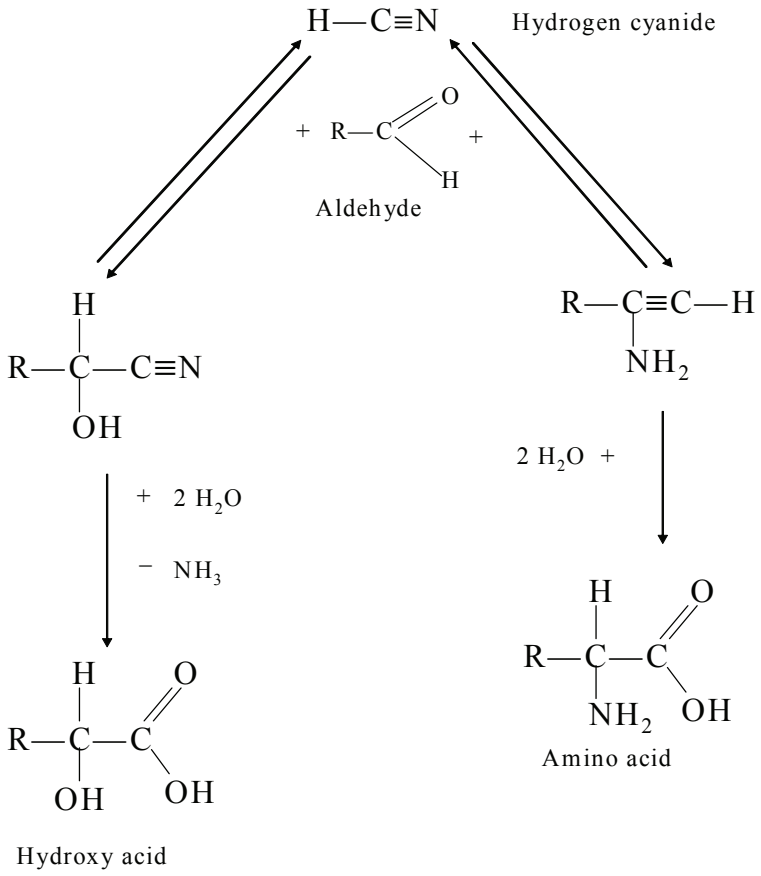
The cyanogenic polydesmids *Oxidus gracilis* and *Harpaphe haydeniana* (Section 4.1.3) can convert (externally secreted) HCN to β-cyanoalanine, asparagine and thiocyanate (SCN); the conversion to SCN is predominant. Other, non-cyanogenic arthropods have the same HCN detoxifying capability, but may be more sensitive to HCN vapours. Symbiotic or contaminant bacteria may be involved (arthropods are often infested with pseudomonads, for example). Rhodanese is often found in moths and butterflies, presumably enabling them to feed upon cyanogenic plants (Duffey, 1981 and references therein).

Bond (1961a,b) studied cyanide metabolism (detoxification) in the grain weevil (*Sitophilus granarius*) and desert locust (*Schistocerca gregaria*). The thiocyanate (rhodanese) pathway was shown to be absent in both species, as was, in *S. granarius* only, the combination of HCN and cystine to 2-imino-4-thiozolidine carboxylic acid. Immediately after 4 hours of exposure to radiolabelled HCN (8 mg/l; 7.1 ppm), *S. granarius* had absorbed 56.7 µg CN/gbw, and 75.5 µg/gbw 4 days later, i.e. the uptake had increased from 33 to 44% during this period. Surviving insects expired a small part of the radioactivity as CO_2 (oxidation), and excreted most (20%) in the faeces as amino acids (with glycine and aspartic acid as probable metabolic products) and cyanocobalamin (as in mammals, Section 7.3).

Abiotic HCN 'metabolism'

Several hydroxy acids and amino acids were formed from a mixture of gases (CH_4, H_2, H_2O and NH_3) subjected for a week to electrical discharges, simulating pre-biotic conditions. The hydroxy and amino acids were produced by condensation of HCN and aldehydes (formed by the electrical discharges) in the Strecker reaction (Miller, 1953, 1957 both cited by Oró and Lazcano-Araujo, 1981), as follows (Figure 13).

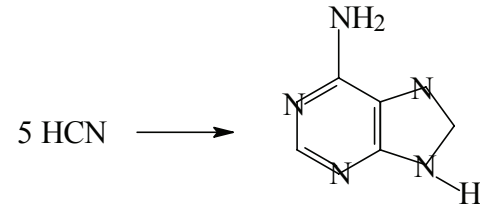
Figure 13: Strecker reaction[a]



[a] R = H for formic acid and glycine, respectively

In addition, the –C≡N triple bond allows HCN to undergo addition reactions, giving higher molecular mass compounds. Thus, cyanide polymerisation may have been involved in the synthesis of important biomolecules like adenine (amino-6-purine) (Figure 14) and other amino acids that make up the DNA helix (Oró, 1960 cited by Oró and Lazcano-Araujo, 1981).

Figure 14: Cyanide polymerisation to adenine



4.3.5 Biodegradation

There are numerous reports of aerobic and anaerobic/anoxic micro-organisms and treatment systems able to degrade cyanide. A variety of enzymatic pathways for cyanide degradation have also been described from aerobic organisms, including hydrolytic pathways leading to formamide, or formate plus ammonia and direct formation of bicarbonate plus ammonia via cyanide oxidation by what is described as a dioxygenase (Fallon *et al*, 1991 and references therein).

This section has two subsections, one describing microbial biodegradation data, and the other one describing experimental data in higher (green) plants. The microbial degradation part is sub-divided into three parts: (i) laboratory-scale and pilot plant experiments investigating kinetics and parameters of aerobic biodegradation, (ii) experiments regarding anaerobic and mixed aerobic/anaerobic or anoxic cyanide biodegradation, and (iii) elimination of cyanide in full-scale wastewater treatment plants.

A great number of studies have been published on this topic some of which have been selected in order to highlight the key findings. Interest in the removal of cyanide by biodegradation had been raised, because of the relatively high cyanide content of a number of industrial effluents (metal finishing, coke production, mining) and the high toxicity of cyanide to surface water ecosystems and standard wastewater treatment plants.

Microbial biodegradation

Aerobic biodegradation - laboratory and pilot plant experiments

Early reports on the work with laboratory-scale units for biodegradation of cyanide-containing wastewater date back to the 1940s and 1950s. Two of those studies are reviewed below, for example (Pettet and Mills, 1954; Gurnham 1955). A review is given by Towill *et al,* 1978.

Depending on the experimental conditions, non-biological effects may contribute to the overall removal of cyanide in biodegradation experiments. Raef *et al* (1977a,b) reported stripping of HCN to be an important removal process. A solution of radiolabelled $K^{14}CN$ (10 mg CN^-/l) was

incubated with cyanide-acclimated activated sludge from a domestic wastewater treatment plant in an aerated micro-fermenter. Up to 50% of the radiolabel was found as $^{14}CO_2$, while the remainder was mainly trapped in the form of volatilised H^{14}CN. Bucksteeg (1960) also observed a contribution of HCN evaporation to the overall removal of cyanide in a sterile trickling filter set up to determine the appearance of ammonia, a degradation product of HCN. The presence in the test medium of certain chemicals which may form cyanohydrins, for example glucose, may also increase abiotic degradation of cyanide (Hope and Knowles, 1991). Ammonia also occurred as a degradation product of HCN, when it was incubated under sterile conditions with certain carbohydrates.

Aronstein *et al* (1994) investigated the removal of radiolabelled cyanide (25 mg/l K^{14}CN; 9.9 g CN^-/l) by a culture inoculated with an aerobic mixed consortium of micro-organisms isolated from the soil of a gas plant and by a culture of a methylotrophic *Pseudomonas* species resistant to cyanide. After 15 days, approximately 60% of the cyanide had been removed in the aerobic mixed consortium culture and 65% in the methylotrophic *Pseudomonas* culture, in both cases approximately 33% thereof by biodegradation and 67% by volatilisation. Almost all (90%) of the ^{14}C label of the biodegraded cyanide was recovered as $^{14}CO_2$.

A percolating filter was gradually adapted to feed concentrations of 1, 2 and 4 mg HCN/l, and in larger steps to 200 mg/l, to achieve cyanide degradation. Almost complete removal of cyanides was observed up to 100 mg/l in the case of KCN and other easily dissociable cyanide salts (zinc and cadmium). At 200 mg/l KCN, the cyanide concentration in the effluent was still approximately 80% lower than in the feed (Pettet and Mills, 1954).

The behaviour of cyanide in a trickling filter fed with municipal wastewater was investigated. Initially, 2 mg CN^-/l (as NaCN or KCN) was enough to affect the trickling filter operation, for example by inhibition of nitrification. After about one week of continuous feed with 2 mg CN^-/l, nitrification returned to normal. When the same experiment was repeated with a non-adapted filter and 30 mg CN^-/l feed, biological oxygen demand (BOD) resumed after 50 to 75 days and nitrate and nitrite re-appeared after about 45 days. After adequate adaptation, 99% removal of cyanide was achieved at a feed concentration of 100 mg CN^-/l. Significant cyanide removal was still observed at 200 mg CN^-/l, though in combination with less efficient BOD removal. In further experiments, degradation of 100 mg CN^-/l was maintained even when the wastewater feed was gradually exchanged to tap water, proving that the trickling filter was using cyanide as its sole energy source. In this experiment, 80 to 90% of the cyanide nitrogen was found in the effluent, mainly as nitrate but also nitrite and ammonia (Gurnham, 1955).

Howe (1965) reported several techniques for the bio-destruction of cyanide wastes. In an experiment with HCN being degraded by activated sludge, which had been acclimatised for two

weeks, 99.8% removal was observed at 35 mg CN^-/l, 98.4% removal at 50 mg/l, 90% removal at 75 mg/l and more than 60% removal at 180 mg/l.

Extensive studies concerning cyanide removal from a feed stream have been performed by Ludzack and Schaffer (1962). They investigated the biodegradation and adaptation behaviour of activated sludge which had been acclimatised to CN^- (as KCN), SCN^- (as KSCN) and OCN^- (as KOCN) in an aerobic bench-scale activated sludge unit co-fed with cyanide solution dissolved in sewage and sucrose (1 g/d). Cyanide removal was close to 100% for most of the time with feed concentrations between 5 and 75 mg CN^-/l. They observed episodes of lower degradation efficiency, especially at the highest feed rate of 75 mg CN^-/l where the degradation rate fell below 80% on one occasion. At 45 and 60 mg CN^-/l these episodes were less pronounced. An increased adaptation period was needed to stabilise the degradation rate at a high level. At a feed concentration of 60 mg CN^-/l, 98 to 100% was removed over an observation period of 18 days, with the exception of a single observation day with a lower degradation rate of around 90%. Generally, the cyanide degradation appeared to be less effective at CN^- concentrations above 60 mg/l. Another finding was that sufficient time had to be given for adaptation of the sludge to increasing cyanide concentrations (3 weeks). Main metabolites were nitrate and ammonia depending on the current respiratory status of the respective sludge. Ammonia was the main metabolite at higher cyanide concentrations. For these experiments the unit was operated at room temperature. When the sludge was cooled to 5°C, the degradation rate initially dropped to 55%, but recovered to 70 to 95% after one week. Experiments with sludge cultures cross-acclimatised to SCN^- and OCN^- demonstrated that the thiocyanate-adapted sludge adapted quickly to cyanide degradation. The cyanate-adapted sludge did not adapt well to cyanide degradation.

Biodegradation of mining (cyanidation) wastewater was investigated, in the laboratory, using aerobic activated sludge units (3 and 10 l) inoculated with soil (containing *Pseudomonas* and other species) from a tailings pond of a mining plant. The batch, fed-batch and continuous experiments lasted 20 to 26 days. The majority of the cyanide in the wastewater was present in the form of complex cyanide salts with less than 10% as free cyanide. WAD (weak acid dissociable) cyanide and total cyanide were almost completely removed in the different experiments. Degradation of the free cyanide was not reported separately. After an initial increase in ammonia, cyanide nitrogen was eventually mostly present in the form of nitrate (Boucabeille *et al*, 1994).

A pilot plant type sequencing batch biofilm reactor (volume 1,900 litres) was operated with a NaCN feed concentration of 15 and 20 mg CN^-/l. Cyanide removal was strongly dependent on the duration of the biodegradation cycle (24, 48 or 72 hours) and on the supply with an additional carbon source (glucose). In the initial experiment with a cyanide feed of 20 mg/l and a cycle time of 72 hours, a cyanide removal of 98.5% was achieved (from 20 to 0.3 mg CN^-/l). Shorter cycle times (48 hours) showed slightly less efficient (average 96%) cyanide removal. Biodegradation

for 24 hours reduced the cyanide removal to 78% on average, while removal of the glucose feed at this stage further reduced the average removal rate to 33% and less, decreasing with each cycle. After re-establishing the glucose feed, the removal rate gradually increased to the original value (still at 24-hour cycles). When the cycle time was finally increased to 48 hours again, almost 100% degradation of cyanide was found in the last 14 cycles. Under the process conditions, 0.5 mg CN^-/l/h was removed with additional glucose and 0.1 mg/l/h without glucose (White *et al*, 2000).

Biodegradation of wastewater from cassava processing was investigated in a laboratory-scale aerobic activated sludge unit (20 l). The sludge had been collected from a local wastewater treatment lagoon and adapted for 20 days on sucrose and cyanide feed. The high chemical oxygen demand (COD) of the wastewater (up to 14,000 mg/l) was reduced by flocculation and coagulation to approximately 2,000 mg/l while cyanide decreased from 400 mg/l to 14 to 18 mg/l. The cyanide concentration in the effluent of the aerobic biodegradation unit was between 2 and 3 mg CN^-/l at the beginning and improved to approx 184 µg/l (≈ 99% removal) after 60 days of operation. The cyanide concentration was further reduced in a final flocculation step to 50 µg/l (Oliveira *et al*, 2001).

A laboratory-scale rotating biological contactor was set up for the treatment of wastewater from the electroplating industry, containing mostly silver cyanide (AgCN). The biological contactor was inoculated with a consortium of bacteria isolated from soil (*Citrobacter* and three *Pseudomonad* species). During the adaptation phase of the biological contactor, the initial hydraulic retention time of 54 hours could be reduced and stabilised to 10 hours after 77 days. The optimised temperature was 35°C and the pH 6.5. At 54 hours initial hydraulic retention (maximum cell density) a removal of more than 99.5% of AgCN in the wastewater was achieved within 24 hours. The influent concentration was 5 ± 1.5 mg CN^-/l with sugar cane molasses added as a carbon source (≈ 180 mg/l glucose). The effluent concentration was 0.03 ± 0.01 mg CN^-. At the same time, silver was removed by > 75% (Patil and Paknikar, 2000).

Cell-free protein extracts from cyanide-adapted *Pseudomonas putida* have been shown to contain enzymes which produce ammonia when incubated with cyanide and related chemicals (cyanates, thiocyanates, formamide). Incubation of samples from cyanide-containing waste streams (13 - 138.5 mg CN^-/l) also resulted in the release of ammonia. The cell-free system was suggested as an alternative to bioreactors in the case of high cyanide concentrations because it was less sensitive to cyanide poisoning (Babu *et al*, 1996). In an earlier paper, the same authors described a cyanide-tolerant strain of bacteria (*P. putida*) isolated from soil. Conversion of cyanide to CO_2 and NH_3 was observed when the bacteria, which had been adapted to 100 mg/l NaCN, were incubated with 100 to 400 mg/l NaCN. With increasing CN^- concentration an inhibitory effect of cyanide toxicity was observed. A direct quantitative relationship between feed concentrations and metabolic products could not be established, because the amounts of CO_2 and

NH_3 which were generated appeared to be several fold higher on a molar basis than the reported NaCN feed concentration, so that other carbon and nitrogen sources in the culture medium were likely to have contributed to the observed amounts of these metabolites (Babu *et al*, 1992).

Anaerobic and anoxic/oxic biodegradation - laboratory experiments

Certain abiotic degradation processes may also occur in anaerobic biodegradation experiments. Like under aerobic conditions (above), the formation of cyanohydrins and ammonia in the presence of sugars may also occur anaerobically (Hope and Knowles, 1991).

Anaerobic bio-treatment columns (fixed bed of activated charcoal, up-flow stream, hydraulic retention time 41 hours, 35 - 40°C) in the laboratory were able to remove free or weakly complexed cyanide by 80 to 100%, following initial conditioning for 99 days (to exhaust absorptive capacity and build up a stable suspended cell mass). The inoculum was a mix of adapted anaerobic sludge (from another anaerobic reactor fed with ethanol and cyanide) and anaerobic digester sludge (from a municipal sewage treatment plant). The columns were fed with synthetic wastewater containing the cyanides at 100 mg CN^-/l on average (but occasionally higher than 200 mg/l) in combination with phenol, ethanol (and ammonia, simulating mining effluent) and additional methanol (and metal salts simulating cokery effluent) as carbon source. The ratio of effluent/influent cyanide concentrations remained < 20%, except during two periods of lowered gas (CH_4 and CO_2) evolution when cyanide elimination was significantly lower. Effluent concentrations remained mostly above 1 mg/l, and by the end of the experiments between 0.5 and 1 mg/l. Removal of cyanide in the form of stable complexes was less efficient than removal of free cyanide and weak cyanide complexes. Cyanide removal was strongly correlated with biological activity, but the responsible organism(s) could not be identified. Ammonia and bicarbonate were the end products, as judged by radiolabelling, of cyanide degradation under these anaerobic conditions (Fallon *et al*, 1991).

Using a two-stage anaerobic digester, the average reduction of cyanide was 60 to 70%. The sludge was slowly adapted to increasing concentrations of 1 to 100 mg CN^-/l. The first digester stage was constantly agitated and heated to 38°C (100°F), and fed with a sludge-nutrient mixture containing 100 mg CN^-/l. The overflowing liquid fed a second, non-heated digester (Howe, 1965).

In a mixed two-step aerobic/anoxic activated sludge treatment process, KCN (10, 30 and 60 mg CN^-/l) was added to synthetic wastewater containing phenols, thiocyanate and salts, simulating wastewater from a steel mill coke oven. The sludge had been obtained from a municipal wastewater treatment plant and acclimated to the phenolic feed for four weeks, when the anoxic/oxic unit was set up and fed with cyanide (60 mg CN^-/l) and thiocyanate. After about

3 months of adaptation, the overall removal of cyanide was 96 to 99%. At the same time 90 to 96% of thiocyanate and 85 to 93% of total organic carbon were removed, 70 to 77% thereof in the anoxic step (i.e. used for denitrification). The mean total residence time of 15, 30 or 45 days had a significant influence on total organic carbon and cyanide removal. At 15 days, the final effluent concentration was 0.9 mg CN^-/l, compared to 0.3 mg/l at 45 days (Richards and Shieh, 1989).

Cassava effluent may contain cyanide in concentrations up to 400 mg CN^-/l combined with a high COD content due to suspended starch residues. Several studies have been performed to investigate the possibility of biodegrading wastewater from cassava processing anaerobically.

In parallel to the aerobic biodegradation experiments described above, Oliveira *et al* (2001) investigated treatment of cassava meal processing wastewater in a two-step anaerobic/aerobic reactor (continuous flow 0.4 l/h, 20 l reactor volume for both units). COD removal was approximately 89% and cyanide removal 97% (influent 2.1 mg/l, effluent 0.06 mg/l). Due to its simplicity and higher overall COD and cyanide removal rate, the authors favoured the one-step aerobic process.

Anaerobic biodegradation of cyanide and COD removal was demonstrated in a single-step methanogenic reactor fed with wastewater from cassava production. The reactor was inoculated with sludge containing adapted micro-organisms associated with cassava roots. Cyanide degradation was sensitive to pH (optimum pH > 5) and hydraulic retention time (optimum 4 d). More than 99% degradation was achieved under optimum conditions, reducing a cyanide concentration in the feed of 200 mg/l to less than 0.5 mg/l in the effluent. In another experiment, metal refining wastewater was shown to be biodegraded under similar conditions. Cyanide degradation was sensitive to the presence of starch as a carbon source. When the starch feed was reduced to less than 4 g/l, cyanide degradation deteriorated. It could only be re-established by increasing the starch concentration again and interrupting the cyanide feed for one week (Siller and Winter, 1998).

In a study with wastewater from a flour and cassava processing plant, a continuous-flow, hybrid (stirred, upflow sludge blanket plus fixed bed) reactor was operated at room temperature for 74 days. The study allowed for pH adjustment between the two stages. The COD of the influent at four sections of the plant was 7,500, 9,000, 11,000 and 14,000 mg/l. In the experiment reported, free cyanide in the influent varied between less than 5 and 30 mg/l. Nearly 98% of free cyanide and 96% of the COD was eliminated, accompanied by the formation of biogas (80% methane). In the first, acidogenic part of the reactor, the level of free cyanide was already decreased by 90% (Paixao *et al*, 2000).

A continuous three-stage anaerobic and anoxic/aerobic reactor with an overall hydraulic retention time of 10 d was fed with synthetic wastewater containing phenol (1,000 mg/l), high COD (3,000 mg/l) and CN^- (30 mg/l), SCN^- (400 mg/l), and NH_4^+-N (600 mg/l) and well-acclimated heterogeneous microbial cultures. After an initial phase of adaptation (25 d), the primary anaerobic stage removed up to 80% of cyanide during the steady-state phase (day 25 - 50), thereby detoxifying the feed for the subsequent stages. Complete thiocyanate removal and denitrification could be achieved in the anoxic stage by utilising phenol as an internal source of carbon. Nitrification efficiency of 93% was obtained in the aerobic reactor. The overall efficiency of cyanide removal was 98%, resulting in effluent concentrations of 0.2 ± 0.13 mg CN^-/l (Chakraborty and Veeramani, 2002).

Biodegradation of cyanide by bacterial nitrogenase, and cyanide intoxication of that system, has been investigated by Jaeger and Dotterweich (1986) using sludge obtained from the sediment of the River Alster in Hamburg, Germany. Although strictly speaking not a biodegradation study, this paper has been included here, because it shows that an anaerobic bacterial consortium from river sediment has the potential to biodegrade cyanide. Under anaerobic conditions, nitrogenase activity (as measured by acetylene reduction), was inhibited by cyanide concentrations of 100 to 150 mg CN^-/kg (wet weight). Optimum HCN removal was observed at concentrations of 5 mg CN^-/kg (wet weight).

Cyanide removal in full-scale wastewater treatment plants

According to a long-term survey of cyanide emissions in the effluents of wastewater treatment plants in the Chicago (USA) area from 1973 to 1977, the majority of CN^- was discharged as (mostly iron and/or cobalt) complexes (64 - 83% in raw sewage; 64 - 87% in final effluents). Compared to the corresponding influent concentrations, ranges of removal rates of total cyanide were 25 to 91%. The content of free cyanides and the concentrations of cyanides in general tended to be higher where the wastewater was mostly of industrial origin while domestic wastewater had generally lower cyanide concentrations with an even smaller fraction of free cyanide (Table 11) (Lordi *et al*, 1980).

Table 11: Cyanide removal in municipal wastewater treatment plants I (Lordi *et al*, 1980)

Type of wastewater / Treatment plant	Concentration of total cyanide [a] (µg CN^-/l)		Removal [a] (%)	Composition of cyanides [b] (%)			
				Influent		Effluent	
	Influent	Effluent		Complex	Simple	Complex	Simple
Industrial [c]							
Calumet	175	79	55	68	32	72	27
Northside	89	8	91	64	36	77	23
West-Southwest	126	41	81	71 72	29 28	64	36
Hanover Park	5	5	0	74	26	83	17
Residential							
Lemont	22	16	25	83	18	87	13

[a] Average during sampling period March to April 1974
[b] Average during sampling period November 1974 to February 1975
[c] Mostly

Cyanide removal in wastewater treatment plants from the same region in Chicago were reported by Kelada (1989). With generally lower cyanide concentrations, the removal rates were still of the same order of magnitude. Concentrations of WAD cyanide in the effluents of the treatment plants were lower than 10 µg/l in all cases. As with the earlier survey above, the presence of cyanides in the wastewater was mostly associated with industrial origin, while their concentrations in residential waste were lower (Table 12).

Table 12: Cyanide removal in municipal wastewater treatment plants II (Kelada, 1989)

Type of wastewater / Treatment plant	Concentration of total / WAD cyanide (µg CN^-/l)		Removal, total / WAD (%)	Composition of cyanides (%)			
				Influent		Effluent	
	Influent	Effluent		Complex	Simple	Complex	Simple
Industrial [a]							
Plant 1	63 / 15	29 / 5	46 / 67	76	24	83	17
Plant 2	95 / 16	12 / 5	87 / 69	82	18	58	42
Industrial-residential							
Plant 3	67 / 46	13/ 7	81 / 85	31	69	62	38
Residential [a]							
Plant 4	20 / 4	5 / 3	75 / 25	85	15	40	60

[a] Mostly

An rotating biological contactor process was developed for the treatment of cyanidation wastewater from a mining company, co-fed with soda ash and phosphate. The (aerobic) process not only oxidised free and complexed cyanides, including the stable iron complexes, but also thiocyanate and the oxidation by-product ammonia. The bacterial inoculum had been isolated from a waste stream of the mining plant and gradually and specifically acclimated to the waste. Results of other laboratory-scale biodegradation units were also presented (Mudder and Whitlock, 1984) (Table 13). The parameters of the full-scale plant have been published in Scott (1985).

Table 13: Biodegradation of cyanide mining wastewater (Mudder and Whitlock, 1984)

	Total cyanide		WAD cyanide	
	Concentration (mg CN^-/l)	**Average removal (%)**	**Concentration (mg CN^-/l)**	**Average removal (%)**
Influent	6.50 ± 2.10	-	4.10 ± 1.30	-
Rotating biological contactor pilot plant	0.35 ± 0.10	95	0.05 ± 0.02	99
Laboratory activated sludge unit	0.27 ± 0.27	96	0.06 ± 0.06	99
'Biotower' 22 ft (6.7 m)	0.87 ± 0.30	87	0.08 ± 0.04	98
Biological aerated filter	1.00 ± 0.60	85	0.06 ± 0.02	99
Influent full scale plant	2.00	-	-	-
Full scale rotating biological contactor plant	0.30	85	0.02	-

Biodegradation by higher plants

As shown earlier (Section 4.3.4), some plants are able to metabolise cyanide. The following experimental data demonstrate that plants may biodegrade cyanide in water and soil.

Water

A microbial "plant marsh filter" system consisting of rocks supporting the growth of Southern bulrush (*Scirpus californicus*, an aquatic macrophyte) was shown to remove KCN and potassium ferricyanide efficiently. In 8 to 14 repeated experiments, average KCN feed concentrations of 3.0 ± 1.8 mg/l (range 0.2 - 6.35 mg/l) or 12.6 ± 10.3 mg/l of potassium ferricyanide (3.5 - 32.8 mg/l) were greatly reduced within 24 hours, to less than 0.2 mg/l for both of the two cyanide species (Wolverton and Bounds, 1988). This represents a removal of > 90% of the cyanide concentration in the influent.

Cyanide degradation by water hyacinths (*Eichornia crassipes*) was investigated in batch tests, using solutions containing 3 to 300 mg CN^-/l dissolved in water with added sewage sludge. Water hyacinths were more efficient at removing free cyanide during the first 8 hours, compared with untreated controls without plants. Gold mill synthetic effluents containing free cyanide (9 to 20 mg/l), thiocyanate (14 to 23 mg/l), and metallo-cyanides (iron, copper and zinc) were fed to a continuous laboratory-scale unit (6 l/h) to confirm the ability of water hyacinth to degrade free cyanide and remove zinc and small amounts of iron. Cyanide was removed by up to 56% while

copper and thiocyanate remained untouched in the solution. In the effluent tests, the plants suffered from obvious signs of toxicity (discoloration, death). According to the results, with regard to gold mill effluents water hyacinth is only suitable for use in conjunction with other cyanide wastewater treatments (Granato, 1993).

In basket willows (*Salix viminalis*), the removal of KCN from nutrient solutions (1 to 50 mg CN^-/l) was almost complete as judged by free cyanide concentrations; 5 mg/l or higher was ultimately lethal to the plants. In the course of the experiment, CN^- was found in the plant tissues in free and complex form. In a control experiment without plants, no loss of CN^- from the solution was found (Trapp *et al*, 2001a). The mass balance of relative amounts absorbed, volatilised and biodegraded was reported in another paper (Trapp *et al*, 2001b). Additional experiments investigated the ability of plant parts (leaves and roots of basket willow, elder or balsam poplar, birch roots, leaves and flowers of roses) to remove cyanide from test solutions in closed bottle tests. In all cases, removal of cyanide from the solution was observed, willow leaves and roots being the most effective and rose petals being the least effective. Willow leaves removed 90% of cyanide from a 1 mg/l solution in 18 hours and 82% from a 10 mg/l solution in 168 hours (7 days) (Larsen *et al*, 2004).

A wide variety of Chinese flora (28 plant species) was more or less capable of removing cyanide from test solutions. Removal rates at a concentration of 1 mg CN^-/l were between approximately 71% and more than 99% in 28 hours. Chinese elder (*Sambucus chinensis*) showed the highest rate of cyanide removal at 8.77 mg CN^-/kg/h (Yu *et al*, 2004)

Soil

The removal of free and complex cyanide from contaminated soils has been investigated using gas work soils (Trapp *et al*, 2001a). In these soils, cyanide is present mostly in complex form with only a small fraction as free cyanide. The initial results appear to be insufficient for a quantification of the removal of cyanides from soil by plants. However, the uptake of cyanide in soil by plants indicates that phyto-remediation of cyanide-contaminated soils may be useful.

Summary of plant biodegradation

Plants are able to remove cyanides from water, and some species, e.g. basket willows or Chinese elder, seem to be efficient. Initial data indicate that cyanide-tolerant plants may be used for the remediation of cyanide-contaminated soils (e.g. around decommissioned city gas plants). Laboratory experiments indicate that the β-cyanoalanine pathway seems to be the most important

metabolic pathway in plants, but it is not entirely clear whether this is the case in the experiments described above.

4.3.6 Summary of metabolism and biodegradation

Cyanide can be metabolised by a wide variety of organisms, including bacteria, fungi, arthropods and plants, following a number of different pathways (Section 4.3.4).

Cyanide is problematic in non-adapted aerobic biodegradation processes because of its toxicity to activated sludge. Initial effects in non-adapted sludge may be found at 0.1 to 0.2 mg/l free cyanide (Section 6.1.4). At non-toxic concentrations, free or weakly complexed cyanide has been shown to be removed in standard municipal treatment plants. Observed effluent concentrations were in the range of 3 to 8 µg CN^-/l.

Adapted sludge can almost completely biodegrade up to 100 mg free CN^-/l. This has been put into practice in treating mining effluents with a total cyanide content of 2 mg/l (≈ 67% of which was WAD cyanide). Approximately 85% of the cyanide content was removed in the plant, the effluent containing approximately 20 µg/l of WAD cyanide.

Concentrations of cyanide, which are non-toxic to the particular inoculum, can be readily biodegraded both aerobically and anaerobically. Aerobic degradation yields carbon dioxide and ammonia (which may be further converted to nitrate or nitrite). Anaerobic biodegradation yields ammonia and methane. Degradation of cyanides in sewage treatment plants depends on the availability of adapted organisms. Sudden high levels of cyanide in these sewage plants may lead to a loss of viability, while fully adapted sludge may tolerate and degrade up to 100 to 150 mg CN^-/l with a high degree of efficiency.

Plants (vegetation) are able to remove cyanides from water and/or soil, and some species, e.g. basket willow or Chinese elder, seem to be efficient. Initial data indicate that cyanide-tolerant plants may be used for the remediation of cyanide-contaminated soils (e.g. around decommissioned city gas plants). Laboratory experiments indicate that the β-cyanoalanine pathway seems to be the most important metabolic pathway in plants, but it is not entirely clear whether this is the case in the experiments described above.

4.3.7 Bioaccumulation

Based on its physico-chemical properties (Table 1: log K_{ow} = –0.25), HCN is not expected to bioaccumulate in aquatic organisms. HCN is either quickly metabolised or the organism dies (Callahan *et al*, 1979).

4.4 Overall assessment of environmental fate

This section summarises all environmental sources (Section 4.1), distribution (Section 4.2) and fate processes in air, water and soil (Section 4.3).

4.4.1 Mackay level III modelling

Global

A chemical released into the environment will be distributed over all environmental compartments (air, water, soil and sediment) by means of physico-chemical and biological processes such as evaporation, deposition, adsorption, desorption, sedimentation, resuspension, degradation and biodegradation. The Mackay equilibrium criterion (EQC) Level III model contains mathematical formulations for all these processes. In combination with input rates of the substance and advection of water and air for an environmental compartment of 316 km^2, the steady-state levels in the compartments for this hypothetical environment can be calculated. The steady-state concentration ratios between the compartments are not only controlled by partitioning (due to fugacity) alone (as in level I and II of the EQC model, Section 4.2), but also by the resistance to transfer between the compartments and the extent of degradation in the different compartments (Mackay *et al*, 1996). Thus, at steady state, the concentration ratios of mass transfer are dependent on whether the input is to air or water (Table 14).

Table 14: Input of HCN to air and water at 25°C following EQC level III modelling (ten Berge, 2004a)

Compartment	Input to air (%)	Input to water (%)
Air	91.31	7.77
Water	7.97	92.0
Soil	0.707	6.01×10^{-2}
Sediment	1.33×10^{-2}	0.154
Half-life in air	8,556 h (356.5 d)	
Half-life in water, soil	360 h (15 d)	
Half-life in sediment	1,440 h (60 d)	

These results show that cyanide discharged in surface water will remain mainly in the water compartment at compartmental transfer equilibrium and the partition equilibrium will never be achieved. The same is true for emissions to air. These results were confirmed using EpiWin; this uses essentially the same calculations (US-EPA, 2000; ten Berge, 2004b).

It should be realised that in the 'unit world' according to Mackay, the air and water compartments are flushed with air and water, resulting in advective residence times of 100 and 1,000 hours, respectively. Furthermore, the surface area of the environmental compartments is divided between 90% soil and 10% water. This is very different, on a global scale, from the land-water ratio of the earth. Therefore, the similar HazChem model (ECETOC, 1994) was used to account for 70% water and 30% soil, as for the earth's surface area. The height of the air compartment was scaled up to 5,000 m and the depth of the water compartment to 200 m, while advection of water and air was set to zero, because no water or air can escape the planet Earth. The use of these 'real world' dimensions showed an overall residence time of 2,310 hours (3.3 months). The release rate of HCN required to achieve a steady-state level in air of 0.2639 μg/m^3 (243 ppt) of HCN in this real world required an annual input of 3.1 Mt HCN (ten Berge, 2004c).

The real world EQC level III values (3.3 months and 3.1 Mt HCN/y) are in line with the data of Singh *et al* (2003) and Li *et al* (2003), who studied the sources and sinks of HCN over the Pacific Ocean (Section 4.3.1). Those two authors estimated mean overall atmospheric lifetimes of 5 and 6.2 months, respectively and global total sources of 2.1 and 1.60 Mt HCN/y, respectively, mainly from biomass burning (Section 4.1.1, 4.4.2).

4.4.2 Mass balance (atmospheric budget of hydrogen cyanide)

Table 15 summarises the available estimates of HCN emissions from biomass burning and other sources (Section 4.1).

Most biomass burning estimates have been derived from emission ratios with respect to CO measured in controlled laboratory fires, in prescribed burns or in real forest and savannah fires. The variability of the emission ratios, due to different type and heterogeneity of fuel, burning conditions and nitrogen content, may explain the range of derived estimates. For example, Yokelson *et al* (1977) found relatively high HCN emissions from smouldering fires and organic fuels. The derived estimates typically lie in the range of 0.5 to 1.3 Mt N/y (1.0 - 2.5 Mt HCN/y) (Singh *et al*, 2003). The relatively high HCN/CO ratios measured in African fires (Yokelson, 2003) suggest that the global atmospheric input of HCN may be near the upper limit of this range.

Table 15: Global atmospheric input of HCN from different sources, by decreasing magnitude (adapted from Li *et al*, 2003 and Singh *et al*, 2003)

Source (Section of report)	Mt N/y	Mt HCN/y [a]	Basis of calculation (emission ratio)	Reference
Biomass burning (4.1.1)				
Total	1.4 - 2.9	(2.7 - 5.6)	Modelling	Li *et al*, 2000
	0.64 - 3.18	(1.23 - 6.13)	*In vitro* (controlled laboratory) fires	Lobert *et al*, 1991 cited by Li *et al*, 2003
	0.63	(1.22)	*In situ* (plumes) over Pacific Ocean	Li *et al*, 2003
	0.5 - 1.3 [b]	(1.0 - 2.5)	*In situ* (plumes) over Pacific Ocean	Singh *et al*, 2003
	0.5 [c]	(1.0)	Literature review	Andreae and Merlet, 2001 cited by Singh *et al*, 2003
	0.4 - 1.2 [d]	(0.8 - 2.3)	*In situ* (plumes) over Pacific Ocean	Singh *et al*, 2003
	0.37 - 1.89	(0.71 - 3.65)	*In vitro* (controlled laboratory) fires	Lobert *et al*, 1990 cited by Li *et al*, 2003
	0.26 [c]	(0.50)	Literature review	Andreae and Merlet, 2001 cited by Li *et al*, 2003
	0.2 - 0.6 [c]	(0.4 - 1.2)	*In vitro* (controlled laboratory) fires	Holzinger *et al*, 1999 cited by Singh *et al*, 2003
	0.1 - 0.3 [c]	(0.2 - 0.1)	*In vitro* (controlled laboratory) fires	Holzinger *et al*, 1999 cited by Li *et al*, 2003
Residential charcoal	0.2	(0.4)	*In situ* (plumes) over Pacific Ocean	Li *et al*, 2003
Biofuels	0.21	(0.41)	Literature review	Andreae and Merlet, 2001 cited by Li *et al*, 2003
Tobacco smoking	-	0.000219	Global, 100 µg/cigarette	This report (Section 4.1.1)
	-	(0.006 - 0.34) [e]	USA data	Fiksel *et al*, 1981 cited by ATSDR, 1998
Industrial sources (4.1.2)				
Total including fossil fuel	0.0016 [f]	(0.0031)	US-TRI data	Singh *et al*, 2003 (Section 4.3.1)
	< 0.05	(< 0.10)	By difference	Singh *et al*, 2003 (Section 4.3.1)
	< 0.001	(< 0.002)	Review	ATSDR, 1998
	-	0.00157	EU data	EPER, 2004

Table 15: Global atmospheric input of HCN from different sources, by decreasing magnitude (cont'd)

Source (Section of report)	Mt N/y	Mt HCN/y [a]	Basis of calculation (emission ratio)	Reference
Industrial sources (4.1.2) (cont'd)				
Primary production		No data		
Use and processing	-	0.022	10% of NaCN in mining	Korte and Coulston, 1998
Waste disposal		No data		
Secondary production		0.000345		US-EPA, 2004a
		0.00102		EPER, 2004
Accidental releases		No data		
Biogenic sources (4.1.3)				
Total	0 - 0.2	(0 - 0.4)	By difference	Singh *et al*, 2003
Other sources (4.1.4)				
Car exhaust	0.04	(0.08)		Lobert *et al*, 1991 cited by Li *et al*, 2003
	< 0.02 [g]	(< 0.04)	Measurements over Los Angeles	Singh *et al*, 2003
Accidental fires		No data		
Volcanoes and lightning		No data		

Table 15: Global atmospheric input of HCN from different sources, by decreasing magnitude (cont'd)

Source (Subsection of Chapter 4.1)	Mt N/y	Mt HCN/y [a]	Basis of calculation (emission ratio)	Reference
Published totals				
	1.4 - 2.9	2.7 - 5.6		Li *et al*, 2000
	1.1	2.1		Singh *et al*, 2003
	0.83	1.60		Li *et al*, 2003
	0.47	0.91		Andreae and Merlet, 2001 cited by Li *et al*, 2003
	0.1 - 0.3	0.2 - 0.6		Cicerone and Zellner, 1983

[a] Converted values
[b] Normalised to CO
[c] Different secondary quotations that could not be resolved (original literature not available)
[d] Normalised to CH_3Cl
[e] Reported as 13,000 - 750,000 pounds (Section 4.1.1)
[f] HCN for 55% air discharge
[g] Including CH_3CN

During the international Southern Africa regional science initiative (SAFARI) campaign held in 2000, it was noted that biomass burning may inject an amount of trace gases into the troposphere that is almost as large as the contribution of fossil fuel burning (Yokelson, 2003).

Assuming a global biomass burning source of 0.9 Mt CH_3Cl/y (87% biomass fires and 13% biofuels/charcoal [based on recent literature data]) and excluding urban influences (C_2Cl_4 < 5 ppt), the global emission of HCN from biomass burning was 0.8 ± 0.4 Mt N/y (0.8 - 2.3 Mt HCN/y). The calculations used enhanced emission ratios for the free tropospheric layer (from plumes due to fires) and marine boundary layer (from biofuel/coal emissions) (Section 4.1.1). Most (> 90%) of the emissions were from fires and a small fraction (10%) from biofuels/charcoal. A similar analysis, using emission ratios with respect to CO (600 Mt/y, 74% from biomass fires and 26% from biofuels/charcoal), resulted in a biomass burning source estimate of 0.9 ± 0.44 Mt N/y (1.0 - 2.5 Mt HCN/y). The figure was less precise because non-biomass burning sources might contribute to CO. The authors cautioned that both estimates were relative and would be directly affected by uncertainties in the global biomass burning emission of CO and CH_3Cl. Emissions from industry and fossil fuel combustion were comparatively small (< 0.05 Mt N/y) (Singh *et al*, 2003).

The calculated total atmospheric burden of HCN of 0.44 Mt N/y and lifetime of 5.0 months (both above) implied a global input of some 1.1 Mt N/y (2.1 Mt HCN/y). Thus, although the global budget was roughly in balance and biomass burning can account for most of the global HCN emissions, the possibility of additional primary biogenic or secondary sources of up to 0.2 Mt N/y (0.4 Mt HCN/y) (in combination with further minor sinks) could not be ruled out (Singh *et al*, 2003).

Li *et al* (2003), adapting their global model (Li *et al*, 2000; Section 4.3.1) to TRACE-P concentration profiles, calculated a HCN input of 0.63 Mt N/y (1.22 Mt HCN/y) from biomass burning and urban (residential coal burning) plumes, i.e. smaller than previous estimates (Li *et al*, 2000). The estimated global ocean uptake of HCN was 0.73 Mt N/y (1.41 Mt HCN/y) and the sink from reaction with ˙OH 0.1 Mt N/y (0.2 Mt HCN/y). These results point to a net global HCN deficit.

Compared to total HCN releases (2.7 - 5.6 Mt HCN/y, predominantly from biomass burning), airborne emissions from the mining industry are relatively small. Korte and Coulston (1998) estimated the amount to 22 kt HCN/y. This corresponds to 0.022 / 2.7 - 5.6 Mt HCN = 0.4 to 0.8% of the total. Similarly, HCN released from biofuels, residential charcoal and biogenic sources may provide 7 to 15% of the total from biomass burning. Tobacco smoking and industrial sources are negligible.

In the case of biomass burning, according to Table 15, 1.1 - 2.5 Mt HCN/y is absorbed by the oceans. Compared to total HCN input of 2.7 - 5.6 Mt HCN/y, approximately 40% of the total amount of HCN emitted through biomass burning is absorbed by the oceans. This is also true for all of the other HCN sources.

4.5 Evaluation

Any local emission of HCN from whatever source is distributed to the global atmosphere and enters the global HCN distribution and fate pathways. Global atmospheric movement can distribute any HCN over long distances and with a high dilution effect. Therefore it must be considered that HCN emissions from point or diffuse sources are quickly diluted and transported in air without negative effect on life forms and the environment. It is very likely that all HCN enters the global HCN input/output system which has a major sink in the global ocean systems.

There is no information in the literature of any negative effects from HCN in the atmosphere on life forms or on the environment. This also reinforces the assumption that HCN emissions can only have importance in regard to local impacts, mainly in regard to worker health considerations. Potential local impact of HCN emissions to air can be expected from direct exposure of humans to volatile HCN or from smoke released from smouldering biomass or smouldering nitrogen-containing plastics or textiles, at a short distance from the source.

Depending on the environmental conditions, the fate of cyanide in natural waters can be very complex. The major degradation process is metabolism by micro-organisms. After oxidation to cyanate, abiotic hydrolysis to ammonia and carbon dioxide may contribute to cyanide degradation After complexation of cyanides with metal salts, adsorption to sediment may occur.

The fate of cyanides in soil is the result of a complex interaction of volatilisation, absorption, complexation and biodegradation, and depends on the balance of these processes in a particular soil environment.

Cyanides can be metabolised by a wide variety of organisms, including bacteria, fungi, arthropods and plants following a number of different pathways. Degradation of cyanides in sewage treatment plants depends on the availability of adapted organisms. While standard municipal treatment plants could tolerate and remove influent concentrations in the range of 3 to 8 µg CN^-/l, fully adapted sludge may be able to degrade 100 to 150 mg CN^-/l with a high degree of efficiency. Some plant species (e.g. basket willow) can remove cyanides from soil and/or water and may be used for remediation of cyanide-contaminated soils.

5. ENVIRONMENTAL LEVELS AND HUMAN EXPOSURE

5.1 Environmental levels

5.1.1 Air

The concentration of cyanide in the troposphere of the northern hemisphere has been reported to range from 160 to 166 ppt (190 ng/m^3) (Cicerone and Zellner 1983; Jaramillo *et al*, 1989).

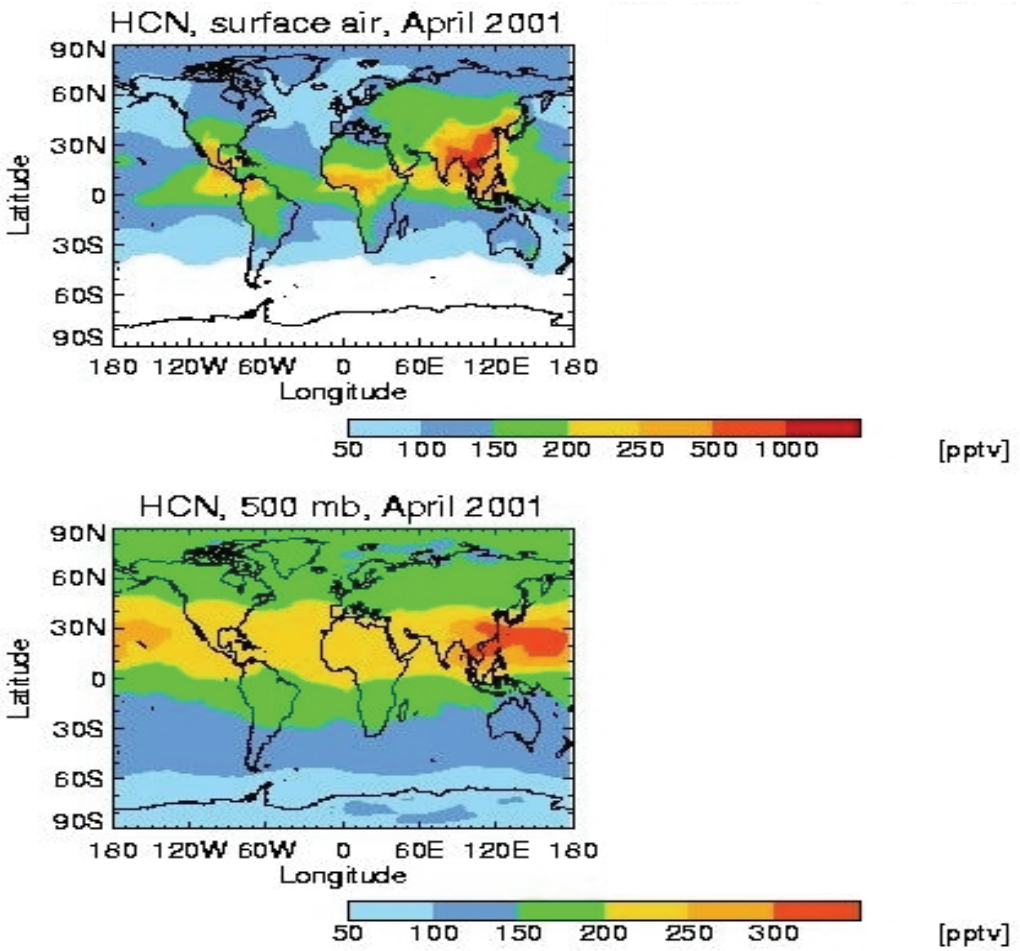
The grand average concentration of HCN in the northern Pacific troposphere (0 - 12 km) was 243 ± 118 (median 218) ppt, as measured during the NASA transport and chemical evolution over the Pacific (TRACE-P) airborne mission (February to April 2001). Enhanced HCN (and CH_3CN) concentrations were largely associated with pollution outflow from the Asian continent. Maximum concentrations were 1,610 ppt. The authors point to strong seasonal variations (Zhao *et al*, 2002 cited by Singh *et al*, 2003) and the high variability of their own data. When the concentration profiles were normalised (to remove scatter) with respect to CO as a generic tracer of pollution or to methyl chloride (CH_3Cl) as a specific tracer of biomass burning, a rapid decline toward the surface of the ocean became evident. Reduced surface concentrations were also apparent directly when applying a further filter for relatively clean atmospheric conditions (CO < 120 ppb, tetrachloroethylene [C_2Cl_4] as a tracer of urban pollution < 10 ppt) (Table 16). The reduced lower tropospheric HCN concentrations in the marine boundary layer were a first indication of a surface sink (Singh *et al*, 2003). These profiles were modelled by Li *et al* (2003) (Section 4.3.1).

Table 16: HCN concentrations over the Pacific Ocean in 2001 (Singh *et al*, 2003)

Altitude (km)	Concentration, all data (ppt)		Concentration, near background air (ppt)	
	Mean ± SD	Median	Mean ± SD	Median
10 - 12	236 ± 77	228	228 ± 52	228
8 - 10	254 ± 92	225	214 ± 36	214
6 - 8	247 ± 66	232	217 ± 41	218
4 - 6	244 ± 70	226	221 ± 53	202
2 - 4	248 ± 117	209	187 ± 65	177
0 - 2	234 ± 161	197	162 ± 58	130
0 - 12	243 ± 118	218	208 ± 53	208

Concentrations of HCN measured in 2001 in background air (defined by CO < 120 ppt and C_2Cl_4 < 10 ppt [Singh *et al*, 2003]) over the North Pacific were 161 ppt at 0 to 2 km and 201 ppt at 2 to 4 km (Jacob *et al*, 2003 cited by Li *et al*, 2003). Simulated global monthly mean HCN concentrations are shown in Figure 15.

Figure 15: Atmospheric HCN concentrations at low and high altitude [a] (Li *et al*, 2003)



[a] Concentrations near the earth's surface and in the middle troposphere at 500 mbar (500 hPa). pptv = ppt (by volume)

Cyanide can be found in ambient air as gaseous HCN and to a smaller extent as particulate cyanide.

There is no information available on HCN contaminated air above tailing ponds.

5.1.2 Water

Krutz (1981) monitored (fortnightly) the concentration of total cyanide ("free and combined") as measured by colorimetry (addition of pyridine-pyrazolone reagent and subsequent extraction of the polymethine pigment with *n*-butanol; detection limit 0.1 µg/l) in small rivers and brooks in the Eifel area in North-West Germany from July 1976 to July 1977. The mean cyanide concentrations ranged from 0.7 to 42.2 µg CN^-/l and varied with space (due to domestic/industrial discharges) and time (e.g. peak emissions from small galvanic industry). The mean level of cyanide in rural watersheds was approximately 3 µg/l, with seasonal variations that were related (by means of different statistical analyses) to blooming algae in spring and decomposing organic matter in autumn and winter. In one natural brook ("Wehebach"), cyanide concentrations were about 0.5 to 1 µg/l, up to 2 to 3 µg/l in full spring and late summer and 8 µg/l in winter. Similarly, in another natural stream ("Nette"), normal cyanide levels were 0.7 to 2.1 µg/l, reaching 5 µg/l in spring and 10 µg/l in autumn.

Data from the National Urban Runoff Program in the USA revealed that 16% of urban run off samples collected from cities contained cyanides at levels of 2 to 33 mg/l (Cole *et al*, 1984).

According to the EPA storage and retrieval (STORET) database the mean cyanide concentrations in most surface waters in USA is less than 3.5 µg/l. Earlier (1980) data indicated that in limited areas the levels were higher, exceeding 200 µg/l (ATSDR, 1998).

Cyanides, reported as HCN, NaCN, KCN, calcium cyanide and copper(i) cyanide, have been detected in surface water samples at 70 out of 1,544 US hazardous waste sites sampled. In ground water samples, they were found at 191 of 419 hazardous waste sites and in leachate from 16 of 52 hazardous waste sites. The median cyanide concentrations of the positive samples were 70 µg/l for surface water, 160 µg/l for groundwater and 479 µg/l for leachate (ATSDR, 2003 cited by IPCS, 2004).

Semi-automatic measurements in the Dutch parts of the rivers Rhine and Meuse demonstrate a decline of total ("free and bound") cyanide levels over recent years (1998 - 2003). In both rivers, the point of entry into the Netherlands showed the highest values in the first two years (Table 17). From the year 2001, the water was analysed by flow-through photometry (RIKZ and RIZA, 2004).

Table 17: Concentration of total cyanide (µg/l) in Dutch rivers

Station (number of samples/y)	1998	1999	2000	2001	2002	2003
Rhine						
Belfeld (12 - 13)	3.3 - 20	0.5 - 2.4	< 0.5 - 2.1	< 3	< 0.5 - 1.4	< 0.5 - 2.1
Lobith (6 - 7)	< 2 - 4.4	< 3	< 3	< 3 - 6	< 0.5 - 1	< 0.5 - 2.3
Meuse						
Eijsden (11 - 13)	1.9 - 11	< 3 - 17	< 3 - 4	< 3	< 0.5 - 1.8	< 0.5 - 1.9
Keizersveer (11 - 14)	< 0.2 - 2.9	< 0.2 - 0.8	< 0.2 - 4.4	< 0.5 - 1	< 0.5 - 1	< 0.5 - 1.6

5.1.3 Soil

Examples of measured cyanide concentrations in polluted soil and groundwater in Europe and USA have been taken from the review by Kjeldsen (1999) (Table 18). Background concentrations are orders of magnitude lower.

Cyanide levels, expressed as HCN, in ground water near sago/starch producing factories in India were 1.2 to 1.6 mg/l (total), 0.1 to 0.9 (ACH) and 0.6 to 1.0 mg/l (free) (Balagopalan and Rajalakshmy, 1998) (Section 4.1.3).

Cyanides have been identified in soil of hazardous waste sites in the USA. The median concentrations of the positive samples were 0.8 mg/kg in the subsurface soil (found at 77 sites of the 124 studied) and 0.4 mg/kg in the topsoil (51 positive sites out of 91 sites) (ATSDR, 2003 cited by IPCS, 2004).

Cyanide-containing wastes (mostly below 2,000 mg/kg) were common in soils at sites of former gas manufacturing plants in the USA. Iron-complexes like ferrocyanide (Prussian blue) comprised over 97% of total cyanides in either weathered or unweathered soils, rather than the more toxic free cyanide forms (Shifrin *et al*, 1996).

Table 18: Total cyanide concentrations in soil and groundwater samples (Kjeldsen, 1999)

Location	Soil (mg/kg)	Groundwater (mg/l)	Reference [a]
Unpolluted			
American sites	< 0.005 - 0.5 [b]		O'Hearn and Kesler-Arnold, 1990
Electroplating workshop			
Dutch site	0.1 - 27,000 [c]		Staritsky *et al*, 1992
Gas works			
British sites A	10 - 5,000 (8 - 280) [d]		Thomas *et al*, 1991
British sites B	50 - 1,800 (3 - 30) [d]		Thomas *et al*, 1991
Danish sites		0.2 - 26 [e]	Kiilerich and Arvin, 1993
Dutch sites	10 - 1,000 [f]	0.5 - 35 [g]	Meeussen *et al*, 1994
Dutch sites		2.9 - 17 (0.013 - 0.15) [d,h]	Meeussen *et al*, 1992
Irish site	(1 - 70) [d]		Byers *et al*, 1994
Salt storage			
Danish sites	10 - 600 [i]	0.07 - 1.5 [i]	Østergaard and Borg, 1994
Gold mine tailing			
American site	1.5 - 23	< 0.7 [j]	White and Markwiese, 1994

[a] Cited by Kjeldsen, 1999
[b] Based on 220 samples, average was 0.2 mg/kg
[c] Range from 340 samples, average was 260 mg/kg
[d] Free cyanide
[e] Based on 190 samples from 33 sites
[f] Range based on samples from soil profiles at 3 sites
[g] Range of maximal values from 12 sites
[h] Samples from 3 sites; reported as 0.11 - 0.67 (0.0005 - 0.0057) [d] mmol/l
[i] Range from 2 sites
[j] High concentration of nitrate in samples, up to 105 mg NO_3^--N/l

5.1.4 Biota

Many edible plants contain cyanogenic glucosides, the concentration of which can vary geographically and from season to season (WHO, 1993). Cyanide-containing foodstuffs and their HCN contents have been reviewed by IPCS (2004). Foodstuffs containing the highest levels of cyanogens include Lima beans (2,000 - 3,000 mg/kg), fruit pits (up to 2,170 mg/kg) and bitter cassava tubers (2,450 mg/kg for dried root cortex). Cassava tubers are perhaps the most significant source of dietary cyanides in humans. Although they can vary widely in their cyanogenic glucoside content, most varieties contain 15 to 400 mg HCN/kg fresh weight (Padmaja, 1995 cited by IPCS, 2004).

5.2 Human exposure levels and hygiene standards

5.2.1 Non-occupational

Non-occupational exposure of humans may occur through inhalation of ambient air, drinking water or food.

Borowitz *et al* (1997) made reference to a number of papers that deal with natural occurrence of cyanide. Blood cyanide levels were reported to be 3 µmol/l in human blood of non-smokers and 7 µmol/l in smokers.

ATSDR (1998) reported estimates of average daily intake from inhalation and drinking water. Based on an atmospheric HCN concentration of 188 ng/m^3 (170 ppt) and a daily inhaled volume of 20 m^3, the acceptable daily intake by inhalation of HCN for the general US non-urban (non-smoking) population was estimated to be 3.8 µg.

Among the general human population, sub-populations with the highest potential for exposures to cyanide include active and passive smokers and individuals consuming foods rich in cyanogenic glucosides. WHO (1992, 1993) concluded that because of a lack of quantitative toxicological and epidemiological information, a safe level of intake of cyanogenic glucosides could not be estimated. However, a level of up to 10 mg HCN/kgbw, as specified in the Codex Alimentarius standard for edible cassava plants (CAC, 1991 cited by WHO, 1993) was not associated with acute toxicity.

Blood carboxyhaemoglobin levels of 433 fire fatalities averaged 44.9% and exceeded lethal (≥ 50%) levels in 195 cases. Cyanide levels of 364 fatalities averaged 1.0 mg/l and exceeded lethal levels (> 3 mg/l) in 31 cases. In victims with cyanide levels above 3 mg/l, the mean carboxyhaemoglobin level was 62.5% (Barillo *et al*, 1994).

5.2.2 Occupational

The principal routes of occupational exposure to cyanides is via inhalation, although skin absorption may be significant under some circumstances, such as when personal protection is inadequate and operators are splashed or when airborne concentrations are very high, e.g. during fumigation operations.

A cyanide production plant is generally designed as a closed system. No emissions of HCN or other gaseous components into the air should take place. Spot measurements of HCN (and NH_3) are carried out in the air of the workplace to ensure that the installation is gas tight. If there is no

detectable concentration level, the installation is regarded as leak-proof. Production workers are not normally required to wear protective clothing against HCN and/or NH_3 gases (Degussa, 2004).

During handling of raw materials and end products in cyanide production plants, formation and emission of HCN and NH_3 cannot be excluded for the whole operation time. Detectors are installed near these activities to trigger an alarm when critical levels are exceeded, e.g. in Germany, the OEL of 10 ppm HCN (Section 5.3) is used. In addition, personal detectors are worn during certain work activities if this is considered necessary (Degussa, 2004).

Workers in certain industries have been reported to be potentially exposed to elevated concentrations of cyanide. The activities involved electroplating, metallurgy, pesticide application, fire-fighting, gas works operations, tanning, blacksmithing, metal cleaning, photo-engraving, photography, and the manufacture of steel, cyanides, adiponitrile and other nitriles, methyl methacrylate, cyanuric acid, dyes, pharmaceuticals or chelating agents (Prohorenkov and Kolpakov, 1978 cited by IPCS, 2004; Philips, 1989 cited by IPCS, 2004; IPCS, 1993).

Limited information on occupational exposure for cyanides is available in the literature.

Levels of cyanide in working environments monitored at different facilities in the US from 1976 to 1982 have been reported as follows. In a plating facility of a national airline, the air concentration was 0.001 to 0.004 mg/m^3 (NIOSH, 1982), in a stripping tank of a plating facility of electrical and electronic company in Virginia, the concentration was 4.3 mg/m^3 (NIOSH, 1976) and in a plating facility in Ohio 1.7 mg/m^3 (NIOSH, 1978) (all references cited by ATSDR, 1998).

Knapic *et al* (1981) reported the maximum airborne concentration of cyanide in a galvanising department of a radio producing plant in the USSR as 1.8 mg/m^3 (1.6 ppm).

El Ghawabi *et al* (1975) reported concentrations of cyanide in 15-minute breathing zone samples taken at three electroplating factories. A total of 36 samples were taken, 12 per facility, and the cyanide levels were between 8 and 12.4 ppm HCN (9.0 - 13.9 mg/m^3), 4.2 and 8.8 ppm (4.7 - 9.9 mg/m^3) and 5.9 and 9.6 ppm (6.6 - 10.8 mg/m^3).

At an electroplating plant in India, concentrations of cyanide in air close to the floor were between 0.2 and 0.8 mg/m^3 (0.18 - 0.71 ppm) with an average of 0.45 mg/m^3 (0.4 ppm). In the breathing zone, levels were between 0.1 (0.09 ppm) and 0.2 mg/m^3 (0.18 ppm), with an average of 0.5 mg/m^3 (0.44 ppm) (Chandra *et al*, 1980).

Leeser *et al* (1990) reported atmospheric HCN concentrations during cyanide salt manufacture between 1 and 3 ppm (1.1 - 3.4 mg/m^3) measured by routine Dräger pumps. Personal monitoring levels ranged between 0.01 and 3.6 mg/m^3 (0.1 - 3.2 ppm).

No cyanide could be detected during production of HCN and NaCN in area samples of air in the production hall, near the distillation column or close to the storage tanks. Concentrations of 0.02 to 0.03 mg CN^-/m^3 (3 - 4 h, personal sampling) were determined during filling of NaCN granulate and 0.21 mg CN^-/m^3 during filling of NaCN powder. During cleaning operations (full personal protective equipment was worn) concentrations were 0.08 to 2.34 mg CN^-/m^3 (1 - 2 h, personal sampling). Area measurements during NaCN saturation revealed concentrations of 0 to 1 ppm (0 - 1.1 mg/m^3), and during HCN storage 3 to 8 ppm (3.4 - 9.0 mg/m^3), in one case 30 ppm (34 mg/m^3) (13 measurements, 1996 to 1998). Filling of train cars (no operator exposure) with NaCN caused levels of 2 to 6 ppm HCN (2.2 - 6.7 mg/m^3) measured by area sampling. The highest concentrations were measured during filter cleaning: 5 to > 150 ppm (5.6 - 169 mg/m^3) (6 area measurements) (Degussa, 2002b).

Although limited occupational exposure data appear to be available for facilities involved in the manufacture and primary processing of cyanides, these sites are regulated as 'top tier sites' under control of major accident hazards (COMAH) regulations. Typically, such sites are fitted with strategically placed hard-wired, alarmed, detection systems for CN emissions to air and water. Excursions are strictly recorded and linked to their authorisations/permits under integrated pollution prevention and control (IPPC) or equivalent regulations (EU, 1996).

In conclusion, the majority of the available occupational exposure data tends to support a picture of low-level background and personal exposure to cyanides in the workplace. The data of El Ghawabi *et al* (1975) suggest that workers employed in 'open system' industries such as electroplating, mining/metallurgy and metal cleaning may be more likely to receive significantly higher exposure than employees in 'closed system' industries such as manufacture of cyanides, adiponitrile and other nitriles, methyl methacrylate, and pharmaceuticals.

5.3 Hygiene standards

Workplace air

Several industrialised countries have adopted occupational exposure limit (OEL) values for airborne concentrations of cyanides (Tables 19 - 21). All OELs include a skin notation because of adverse systemic toxicity by the dermal route (Section 8.1.2).

Table 19: OEL values for HCN [a]

Country [b]	Concentration, 8-h TWA [c]		STEL [c], 15-min		Remark
	(ppm)	(mg/m^3) [d]	(ppm)	(mg/m^3) [d]	
Austria	10	11	40	44	STEL 4 × 30 min
Belgium	-	-	4.7	5	STEL
Denmark	4.7	5	-	-	Ceiling
France	2	(2.2)	10	(11)	VME [c]
Finland	-	-	10	11	-
Germany	10	11	-	-	Ceiling 1 h
	-	-	40	44	-
Italy	4.7	(5.3)	-	-	Ceiling
Ireland	-	-	(8.9)	10	-
Norway	5	(5.6)	-	-	Ceiling
Spain	-	-	4.5	5	-
Sweden	(4.45)	5	-	-	Ceiling
Switzerland	1.9	2	3.8	4.2	STEL 4 × 15 min
Netherlands	10	11	-	-	Ceiling
UK	-	-	10	11	MEL [c]
USA[e] OSHA	10	11			PEL [c]
USA[e] OSHA	-	-	4.7	5	-
USA[e] ACGIH	4.7	(5.3)	-	-	Ceiling
Australia	10	11	-	-	Peak limitation
Canada	4.7	(5.3)	-	-	Ceiling
Japan [f]	5	5.5	-	-	Ceiling

[a] The value may be advisory or official (tentative or legally binding). All OELs include a skin notation

[b] For Europe, see: http://europe.osha.eu.int/good_practice/risks/ds/oel/, including all current EU member and accession states, Switzerland, Iceland and Norway (the site also refers to Australia, Canada and US-OSHA, and international UN agencies)

[c] TWA, time-weighted average; STEL, short-term exposure limit; MEL, maximum exposure limit; PEL, permissible exposure limit; VME, "valeur maximum exposition"

[d] Some agencies use (slightly) different conversion factors based on variations in temperature, pressure and/or normal gas volume. Values converted following Appendix C are given in parentheses

[e] For USA, see: ACGIH (2000), AIHA (2001) and NIOSH (2001)

[f] For Japan, see: JSOH (2000)

Table 20: OEL values for cyanide salts[a] (as CN^-)

Country [b]	Concentration, 8-h TWA [c]		STEL [c], 15-min		Remark
	(ppm)	(mg/m^3)	(ppm)	(mg/m^3)	
Austria		5		20	STEL 4 × 15 min
Belgium		5		-	STEL
Denmark		5		-	Ceiling
Finland		5		10	-
France		5		-	VME [c]
Germany		5		20	Inhalable fraction, STEL 4 × 15 min
Italy		5		-	Ceiling
Ireland		5		-	-
Norway		5		-	-
Spain		-		5	STEL
Sweden		5		-	Ceiling
Switzerland		3.8		3.8	Inhalable dust
Netherlands		5		-	-
UK		5		-	-
USA [d] OSHA		5		-	PEL [c]
USA [d] OSHA		-		25	IDLH [e]
USA [d] NIOSH	4.7	5		-	10 min
USA [d] ACGIH		5		-	Ceiling
Canada		5		10	-
Japan [f]		5		-	Ceiling

[a] The value may be advisory or official (tentative or legally binding). All OELs include a skin notation

[b] For Europe, see: http://europe.osha.eu.int/good_practice/risks/ds/oel/, including all current EU member and accession states, Switzerland, Iceland and Norway (the site also refers to Australia, Canada and US-OSHA, and international UN agencies)

[c] TWA, time-weighted average; STEL, short-term exposure limit; MEL, maximum exposure limit; PEL, permissible exposure limit; VME, "valeur maximum exposition"

[d] For USA, see: ACGIH (2000), AIHA (2001) and NIOSH (2001)

[e] Immediately dangerous for life or health

[f] For Japan, see: JSOH (2000)

Table 21: OEL values for ACH [a]

Country [b]	Concentration, 8-h TWA [c]		STEL [c], 15-min		Remark
	(ppm)	(mg/m^3) [d]	(ppm)	(mg/m^3) [d]	
Belgium	4.7	5	-	-	Ceiling as CN^-
Denmark	1	3.5	-	-	As ACH
Italy	4.7	5	-	-	Ceiling as CN^-
Spain	-	-	4.7	5	As CN^-
Netherlands	1	3.5	10	35	As ACH
USA [e] ACGIH	4.7	5	-	-	Ceiling as CN^-
USA [e] AIHA	2	7.1	5	17.7	As ACH
Canada	4.7	5	-	-	Ceiling as CN^-

[a] The value may be advisory or official (tentative or legally binding). All OELs include a skin notation

[b] For Europe, see: http://europe.osha.eu.int/good_practice/risks/ds/oel/, including all current EU member and accession states, Switzerland, Iceland and Norway (the site also refers to Australia, Canada and US-OSHA, and international UN agencies)

[c] TWA, time-weighted average; STEL, short-term exposure limit; MEL, maximum exposure limit; PEL, permissible exposure limit; VME, "valeur maximum exposition"

[d] Some agencies use (slightly) different conversion factors based on variations in temperature, pressure and/or normal gas volume

[e] For USA, see: ACGIH (2000), AIHA (2001) and NIOSH (2001)

5.4 Public and environmental health standards

5.4.1 Indoor air

No limits for cyanide concentrations in indoor air have been established.

5.4.2 Outdoor air

Russia has established maximum allowable target 'SRV' limit values of 0.015 mg HCN/m^3 monthly and 0.01 mg/m^3 yearly (EEA-STAR database, 2002a).

In the USA, the government of Arizona has proposed air quality standards of 40 µg HCN/m^3 (24-hour average) and 100 $\mu g/m^3$ (1-hour average) (Arizona Department of Health, 1999).

Canada has developed a 24-hour average 'ambient air quality criterion' of 8 µg HCN/m^3 and a half-hour 'point of impingement' standard of 24 $\mu g/m^3$, both based on the central nervous system and thyroid effects of this compound (Ontario Ministry of the Environment, 2004).

5.4.3 Drinking water

The World Health Organisation has set a standard for cyanide in drinking water of 0.07 mg/l (WHO, 2003, 2004).

The EC Directive relating to the quality of water intended for human consumption states a maximum admissible concentration for cyanide of 0.05 mg/l (CEC, 1980). In the EU a new Drinking Water Directive came into effect in 2003, but the (total) cyanide limit stayed at 0.05 mg/l (CEC, 1998).

The 1985 amendment to the Safe Drinking Water Act of 1974 (US-EPA, 1985a,b) proposed a maximum allowable quantity of 0.025 mg/l (as cyanide and its derivatives) in drinking water.

The US-EPA (2004b) established a drinking water standard of 0.2 mg/l for cyanides (as free cyanide), based on "nerve damage or thyroid problems." The limit entails both a non-enforceable maximum contaminant level goal (MCLG, i.e. the level of a contaminant in drinking water below which there is no known or expected risk to health) and an enforceable maximum contaminant level (MCL, i.e. the highest level of a contaminant that is allowed in drinking water). MCLGs allow for a margin of safety and are public health goals. MCLs are set as close to MCLGs as feasible, using best available treatment technology and taking cost into consideration.

5.4.4 Groundwater

In 1994, the Netherlands specified an 'intervention value' of 1,500 µg/l for inorganic cyanide complexes in groundwater (the same value applied to cyanide-free inorganic compounds). An intervention value is the standard above which pollution represents an unacceptable risk to water, humans, animals or plants, and above which there will be serious environmental pollution which will require remediation, either in the short or longer term (EEA-STAR database, 2002b).

5.4.5 Surface water

In Germany, HCN, NaCN, KCN and ACH are not classified as hazardous to the aquatic environment ("Wassergefährdungsklasse 3"). These cyanides are subject to provisional self-classification in accordance with EC rules (Section 2.2) (UBA, 2000).

The EC Directive relating to the quality of surface waters intended for the abstraction of drinking water states that all surface water must not contain concentrations of cyanide greater than 0.05 mg/l (CEC, 1975). The EC Bathing Water Directive used to state that the concentration of

cyanides needs to be checked when it may be present or if the water quality has deteriorated (CEC, 1976). The 1976 Directive is superseded by a new Directive (CEC, 2006a) that refers to the EU Water Framework Directive where cyanides are not specified (EC, 2000).

The UK Environment Agency established an 'environmental assessment level' (environmental quality standard *ad interim*) for the protection of aquatic life in inland, estuary and coastal waters. The standard specifies 1 µg/l and 5 µg/l of free cyanide (HCN and CN) as annual average and maximum allowable concentrations, respectively. The annual average concentration was derived by applying a safety factor of 5 to the lowest concentration causing sub-lethal effects following chronic exposure of fish to cyanide (5 µg HCN/l). The maximum allowable concentration was derived by applying a safety factor of 5 to the lowest credible acute effects concentration (28 µg/l for the rainbow trout, *Oncorhynchus mykiss*). The proposed standards were based on fish data as these appeared to be the most sensitive organisms, and thus should provide adequate protection to all species for short-term exposure (Murgatroyd *et al*, 1998; UK-EA, 2003).

US-EPA (1976) proposed a limit of 5.0 µg/l as free cyanide. More recent US-EPA documentation states, however, that aquatic organisms should not be adversely affected if the 4 day average concentration of free cyanide does not exceed 5.2 µg/l more than once in every three years, and the one-hour average concentration does not exceed 22 µg/l, more than once in every three years (US-EPA, 1985c, 1986a).

The Canadian Environment Agency also set 5 µg/l for free cyanide in water (CCREM, 1987 cited by CCREM, 2006).

5.5 Other standards

In the USA, ATSDR (1998) established a 'minimal risk level' of 0.05 mg CN^-/kgbw/d based upon the NOAEL of 4.5 mg/kgbw/d for reproductive effects in male and female rats (Hébert, 1993).

The US integrated risk information system (IRIS) calculated a reference dose for chronic oral exposure (RfD) of 0.02 mg CN^-/kgbw/d based on a NOAEL of 10.8 mg/kgbw/d in the rat (Howard and Hanzal, 1955) (Section 8.5) (US-EPA, 1986b).

HCN was again reviewed under the US IRIS programme in 1994. A reference concentration for chronic inhalation exposure (RfC) for HCN was estimated, based upon CNS symptoms and thyroid effects observed in the occupational study reported by El Ghawabi *et al* (1975). The perceived low confidence in the key study and in the database as a whole was accounted for by additional uncertainty and modifying factors, including 10 for sensitive human subpopulations,

10 for the lack of a NOAEL, 3 for deficiencies in the database (lack of chronic and multigenerational reproduction studies) and 3 for study duration being less than chronic. This resulted in an overall uncertainty factor of approximately 1,000 and a value of 0.003 mg/m^3 being proposed for HCN (US-EPA, 1994). As of September 2002, HCN and ACH are being re-assessed under the IRIS programme.

Cyanide limits in industrial effluents can vary considerably, depending on, for example, the industry, type of effluent and point of discharge. The International Cyanide Management Institute of the gold mining industry has suggested the following effluent limits: < 50 mg WAD CN^-/l for entry to the tailings pond, < 0.5 mg WAD CN^-/l for entry to receiving waterways and < 0.022 mg free CN^-/l after dilution in the waterway (ICMI, 2005). These limits are similar to World Bank (1995) guidelines for mining discharges: 0.1 mg free CN^-/l, 0.5 mg WAD CN^-/l and 1 mg total CN^-/l, or to some national limits, e.g. Indonesia with 1 mg CN^-/l for effluents discharged to a river system. The World Bank adds that the concentration in the receiving water outside of the mixing zone should not exceed 0.022 mg/l.

6. EFFECTS ON ORGANISMS IN THE ENVIRONMENT

6.1 Micro-organisms

Cyanide toxicity has been investigated in a number of unicellular organisms and micro-biocoenoses (activated sludge). These data are summarised in Tables 22, 23 and 24, and discussed in Sections 6.1.2 to 6.1.4. Data on the toxicity of cyanide to aquatic algae have been summarised and evaluated in Section 6.2.

6.1.1 Mode of action

The principle of cyanide toxicity is similar in all organisms including micro-organisms. The cyanide ion reacts with the central metal ion of metallo-enzymes to form cyanide complexes thus rendering those enzymes non-functional. The primary target in cytochrome oxidases is the central iron ion (Fe^{3+}). Examples of other, cyanide-sensitive enzyme systems are catalase and other peroxidases, myoglobin, nitrite and nitrate reductase and nitrogenase (Solomonson and Spehar, 1981) and CO_2 reductase (Thauer *et al*, 1973).

The respiratory systems of bacteria often present similarities with that of mitochondria. They are membrane-bound and exhibit energy conservation through coupling of the respiratory chain redox reactions to ATP synthesis. One of the major characteristics of bacterial systems is the wide range of redox carriers which may have an oxidase function (namely cytochromes *a*, *b*, *c* and *d*). Combination of up to four of these terminal redox carriers is not an uncommon feature in bacterial membranes, and has been shown in different species (Henry, 1981). Jones (1973) demonstrated that *Azotobacter vinelandii* contains three cytochrome oxidases which can be functionally isolated and distinguished by their quantitatively and/or qualitatively different responses to cyanide.

Different types of oxidases with different sensitivities to cyanide may be present depending on the metabolic status of the culture (growing or static). Pudek and Bragg (1974) investigated the kinetics of inhibition by KCN of growing and static cultures of *Escherichia coli* and found that differant types of cytochrome oxidases had different sensitivities to cyanide.

When cultures were selected towards cyanide resistance, e.g. by the presence of low concentrations of cyanide in the culture medium (Fry and Myers, 1981; Henry, 1981; Skowronski and Strobel, 1969; White *et al*, 1988) and/or an effective cyanide metabolism which might detoxify cyanide (Section 4.3.4), cyanide-resistant micro-organisms tolerated concentrations of 1 mmol CN^-/l (26 mg CN^-/l) and higher.

6.1.2 Bacteria

Table 22 summarises toxicity tests with cyanide (inhibition of growth or respiration) in different species of bacteria and cyanobacteria.

Cyanide concentrations effective at inhibiting growth of bacteria span several orders of magnitude from concentrations as low as 0.0004 mg CN^-/l in *Pseudomonas putida* (16-h EC_3) (Bringmann and Kühn, 1977b) up to concentrations above 1 mg/l in other species (*E. coli*, 4-h EC_{50}) (Loveless *et al*, 1954). Taking into account the substantial metabolic differences between bacterial species, the significant influence of the culture conditions on cyanide toxicity, the variety of endpoints tested and the, in total, small number of published toxicity test results, the relevance of those test data for environmental exposure of bacteria to cyanide is unclear. *E. coli* has also been shown to be able to grow when the Petri dishes were incubated in an atmosphere with 1,000 ppm HCN (1,124 mg/m^3) (McCallan and Weedon, 1940).

Data for resistant strains of bacteria have not been tabulated because those do not represent sensitive populations that necessarily need to be protected. As an outstanding example, a strain of *Bacillus subtilis* isolated from soil has been found to tolerate and biodegrade cyanide concentrations as high as 100 mmol CN^-/l (2,600 mg/l) (Skowronski and Strobel, 1969). Depending on culture conditions, several bacteria have been shown to generate cyanide during certain phases of the culture (Section 4.1.3).

6.1.3 Eucaryotes

The toxicity of cyanide has been tested in a number of protozoa (Table 23).

The toxicity threshold, as measured by the inhibition of culture growth, ranged from 0.013 mg CN^-/l in *Tetrahymena pyriformis* (ciliate) (Slabbert and Maree, 1986) to 1.8 mg/l in *Entosiphon sulcatum* (flagellate) (Bringmann and Kühn, 1978a). These tests are well-conducted and follow standard protocols for microbial toxicity testing. With the relatively wide variety of life patterns between the tested organisms these results are regarded as representative for protozoa.

For fungi, toxicity test data are available with *Fusarium lini* (synonym: *F. oxysporum*) and the yeast *Saccharomyces cerevisiae*, both following non-standard protocols (Table 24).

Table 22: Toxicity of cyanide to bacteria and cyanobacteria

Species	Parameter, biological endpoint	Test substance	Concentration [a]		Remark	Reference	CoR
			(mg/l)	(mg CN^-/l)			
Photobacterium phosphoreum, lyophilisate	5-min EC_{50}, inhibition of luminescence	KCN	(33.3)	13.3	-	Qureshi *et al*, 1982	2e
E. coli, strain B	4-h EC_{50}, growth inhibition (cell count)	NaCN	3	(1.6)	No effect on cell size	Loveless *et al*, 1954	2e
E. coli, strain V29	6-h toxicity threshold, metabolic inhibition (decrease of culture pH)	KCN	0.4 - 0.8	(0.16 - 0.32)	-	Bringmann and Kühn, 1959a	2e
P. putida	16-h EC_3, growth inhibition (turbidimeter)	KCN	0.001	(0.0004)	-	Bringmann and Kühn, 1977b	2e
Clostridium pasteurianum	Partial inhibition of CO_2 reductase	KCN	3.3, 6.5 [b]	(1.3, 2.6)	Complexation of Mo-metallo-enzyme	Thauer *et al*, 1973	2e
Microcystis aeruginosa	8-d EC_3, toxicity threshold, growth inhibition (photometer)	KCN	0.073	(0.029)	-	Bringmann and Kühn, 1976	2e
Microcystis aeruginosa	24 h, 90% lethality (cell lysis)	KCN	20	(8.0)	-	Fitzgerald *et al*, 1952	2e

[a] Converted values in parentheses
[b] Reported as 50, 100 µmol/l

Table 23: Toxicity of cyanide to protozoa

Species	Parameter, biological endpoint	Test substance	Concentration [a]		Remark	Reference	CoR
			(mg/l)	(mg CN^-/l)			
Ciliata							
Tetrahymena pyriformis	NOEC LOEC [b], 8% inhibition 71% inhibition; 5 min, respiratory inhibition (O_2 consumption)	NaCN	0.013 0.025 0.5	(0.007) (0.013) (0.27)	-	Slabbert and Morgan, 1982; Slabbert and Maree, 1986	2e
Microregma heterostoma	28-h EC_3, growth inhibition (turbidimeter)	KCN	-	0.04	-	Bringmann and Kühn, 1959b	2e
Spirostomum ambiguum	24-h EC_{50}, morphology alterations 48-h EC_{50} 24-h LC_{50} 48-h LC_{50}	KCN	-	1.28 1.18 2.14 2.04	-	Nalęcz-Jawecki and Sawicki, 1998	2e
Uronema parduczi	20-h EC_5, growth inhibition (cell count)	NaCN	-	0.27	-	Bringmann and Kühn, 1980a	2e
Flagellata							
Chilomonas paramecium	48-h EC_5, growth inhibition (cell count)	KCN	-	1.2	-	Bringmann *et al*, 1980	2e
Entosiphon sulcatum	72-h EC_5, growth inhibition (cell count)	NaCN	-	1.8	-	Bringmann and Kühn, 1978a	2e

[a] Converted values in parentheses
[b] Lowest-observed effect concentration

Table 24: Toxicity of cyanide to fungi

Species	Parameter, biological endpoint	Test substance	Concentration [a]		Remark	Reference	CoR
			(mg/l)	(mg CN^-/l)			
Fusarium lini	5 h, 10 - 25% inhibition of respiration	KCN	0.1 - 1	(0.04 - 0.4)	After prolonged exposure the oxygen consumption increased above controls (not quantified)	Weiss-Berg and Tamm, 1971	2e
Saccharomyces cerevisiae	4 h, 38% growth inhibition (cell count)	NaCN	125	(66.4)	Increase in cell size	Loveless *et al*, 1954	2e

[a] Converted values in parentheses

The toxicity of atmospheres above KCN solutions has been tested in four species of fungi (*Stemphylium loti*, *S. sarcinaeforme*, *S. botryosum* and *Helminthosporium turcicum*). *S. loti*, a pathogen found on cyanogenic plants, was the most resistant to cyanide-induced inhibition of the growth of germ tubes (Millar and Higgins, 1970). Because of the indirect experimental setup, cyanide concentrations in the agar cannot be derived.

McCallan and Weedon (1940) described the toxicity of airborne HCN to cultures of nine species of fungi. In four cases the LT_{50} (time to kill 50% of the cells) after exposure to 1,000 ppm of HCN (1,124 mg/m^3) was longer than the longest exposure period of 960 minutes (16 h), in the other five species it was between 120 and 720 minutes (2 and 6 h). Sclerotia were less sensitive, when exposed under the same conditions. The relevance of these data for the general sensitivity of fungi to cyanide is unclear.

As in the case of bacteria, some fungi are known to proliferate in cyanide-rich environments and may even generate cyanide during growth at concentrations sufficiently high to impair growth of other fungi and plants (for examples, see Lebeau and Hawn, 1963 or Robbins *et al*, 1950).

6.1.4 Activated sludge

Toxicity of cyanide to activated sludge will be a problem if treatment facilities are likely to face wastewaters containing peak, high concentrations of cyanide. Table 25 summarises the available toxicity tests with activated sludge and sewage.

Exposure to cyanide may be lethal to activated sewage sludge. Peak loading of an experimental sewage treatment with concentrations in excess of 30 mg/l KCN (12 mg CN^-/l) induced 70% inhibition of respiration causing a transient, concentration-related deterioration of the effluent quality. The effect lasted 3 days at 120 mg KCN/l (48 mg CN^-/l) and 4 days at 500 mg KCN/l (200 mg CN^-/l) (Pagga, 1985).

In an international ring test with 24 participating laboratories aimed at standardising a growth inhibition test with sewage sludge, 18 laboratories (6 refused for safety reasons) actually tested cyanide as a possible positive control. The average EC_{20} value for growth inhibition of sewage bacteria was 5.5 mg KCN/l (2.2 mg CN^-/l) and the lowest EC_{20} was 0.24 mg CN^-/l. The average EC_{50} (of 17 laboratories) was 4.9 mg CN^-/l, the lowest EC_{50} was 2.0 mg/l and the highest, 9.2 mg/l (Strotmann and Pagga, 1996).

Table 25: Toxicity of cyanide to activated sludge

Sludge type (wastewater treatment plant)	Biological endpoint, parameter	Test substance	Concentration [a]		Remark	Reference	CoR
			(mg/l)	(mg CN^-/l)			
Municipal	Respiratory inhibition	KCN				Strotmann *et al*, 1992	2e
	EC_{20}, OECD 209 [b] in STRA [c]-medium		0.3	(0.12)			
	EC_{50}, standard		2.3	(0.92)			
	EC_{80}		14.4	(5.75)			
Municipal	Respiratory inhibition	KCN				Strotmann *et al*, 1992	2e
	EC_{20}, OECD 209 in BM [d]-buffer		0.2	(0.08)			
	EC_{50}, pH 7 (phosphate buffer 2 g/l)		0.6	(0.24)			
	EC_{80}, sodium acetate or glucose		6.3	(2.52)			
Municipal	EC_{20}, growth inhibition	KCN	5.5	(2.2)	18 tests	Strotmann and Pagga, 1996	2e
	EC_{50}, 6 h, turbidimetric		12.3	(4.9)	17 tests		
	Minimum EC_{50}		5.0	(2.0)	13 tests		
	Maximum EC_{50}		23.0	(9.2)			
	EC_{80}, mean result		27.7	(11.1)			
Industrial	EC_{50}, respiratory inhibition	KCN	30 - 50	(12 - 20)	ISO 8192	Pagga, 1985	2e
	EC_{50}, inhibition		7.0	(2.8)	BASF “Toximeter”		

Table 25: Toxicity of cyanide to activated sludge (cont'd)

Sludge type (wastewater treatment plant)	Biological endpoint, parameter	Test substance	Concentration [a]		Remark	Reference	CoR
			(mg/l)	(mg CN^-/l)			
Municipal	EC_{50}, growth inhibition	KCN	4.0	(1.6)		Alsop *et al*, 1980	2e
Domestic sewage	NOEC, 10 d	Not stated	-	0.2		Ludzack *et al*, 1951	2e
	LOEC ≥ EC_5			0.3			
	EC_{50}, respiratory inhibition (BOD)			12.0			
Municipal	EC_{75}, 2.5 h, inhibition of nitrogenase activity (oxidation of ammonia/nitrite)	NaCN	0.65 [e]	(0.63)	*Nitrosomonas* and *Nitrobacter*	Downing *et al*, 1964; Tomlinson *et al*, 1966	2e
			0.95 [e]	(0.91)	*Nitrosomonas*		
			1.54 [e]	(1.48)	*Nitrobacter*		
			(mmol/l)	(mg CN^-/l)			
Soil microflora (sludge culture)	Adaptive reaction	KCN	0.0001	(0.003)		Zintgraff *et al*, 1969	2e
	EC_{50}, respiratory inhibition		0.01	(0.26)			
	EC_{100}, inhibition		0.5	(10.5)			

[a] Converted values in parentheses
[b] OECD, 1984a
[c] Short-term respiration assay
[d] Phosphate buffer and sodium acetate or glucose as carbon source
[e] CN^- as HCN

Respiratory inhibition of either domestic sewage or domestic sewage sludge by cyanide was found between 0.08 mg/l and 0.3 mg/l (CN^-). It was concluded that cyanide concentrations in excess of 0.1 mg/l might temporarily damage the capacity of sewage plants to biodegrade wastewater (Ludzack *et al*, 1951; Strotmann *et al*, 1992).

Soil micro-organisms cultured under conditions comparable to activated sludge displayed similar behaviour to activated sludge cultures. At 0.1 µmol KCN/l (0.003 mg CN^-/l) a weak, adaptive reaction was observed. 10 µmol/l (0.26 mg CN^-/l) caused a noticeable respiratory inhibition (delay in the oxygen consumption curve) while 500 µm (10.5 mg CN^-/l) inhibited respiration completely (Zintgraff *et al*, 1969).

The influence of cyanide on nitrification of activated sludge co-fed with ammonia was investigated. Cyanide (as NaCN) inhibited nitrification by 75% at a concentration corresponding to 0.65 mg HCN/l (0.63 mg CN^-/l). The individual metabolic steps by *Nitrosomonas* (nitrite formation) and *Nitrobacter* (nitrate formation) were inhibited by 75% at concentrations of 0.95 mg HCN/l and 1.54 mg HCN/l, respectively. In another experiment, the influence of shock-loading with cyanide on a percolating filter and on a laboratory-scale activated sludge pilot plant was investigated. A concentration of 6 mg HCN/l for 24 hours inhibited nitrification in the percolating filter by 40% with almost immediate recovery after the end of exposure. Nitrification in the activated sludge plant was inhibited almost completely but recovered within 24 hours after the end of exposure (Downing *et al*, 1964; Tomlinson *et al*, 1966).

Sludge biocoenoses can be adapted to tolerate higher cyanide concentrations when cyanide concentrations are gradually increased during an acclimation phase (Howe, 1965).

6.2 Aquatic organisms

A search of the available literature on cyanides revealed an extensive database of aquatic toxicity. The present analysis has been based on 207 original references and 14 major reviews on cyanides, including a search for aquatic effect data on cyanide (HCN, NaCN, KCN or ACH) in the US-EPA Ecotox database (US-EPA, 2003). In total, there were 261 references providing 1,263 data. For 1,128 of these data a concentration was given, including limit values (e.g. < x) and estimations (~). These are recorded in Appendix J. References and data records were categorised into 4 classes of reliability (Appendix B). Only reliable toxicity data (CoR 1 or 2) were considered further.

Historically, environmental quality standards have been set by applying an uncertainty factor to the most sensitive species. In cases where there is a sufficiently robust database, a probabilistic approach using the whole database is preferred to derive a species sensitivity distribution (SSD).

In this report, SSDs are used as a graphical tool to summarise results and extrapolate to a hazardous concentration for 5% of the species tested (HC_5). The HC_5 is regarded as a 'safe' concentration for 95% of the species (Posthuma *et al*, 2002). It is used in the current EU chemical risk assessment paradigm that is based on a generic model representing the freshwater and terrestrial environment of Europe (ECB, 2003).

The ECETOC aquatic toxicity (EAT) database (ECETOC, 1993) has been updated, mainly from publications between 1992 and 2000, to include information on the toxicity to aquatic species in fresh and marine waters. The principal quality criteria for acceptance of data were that the test methods were well described and the toxicant concentrations measured. On this basis, 178 (33%) of the 537 papers examined were deemed suitable for inclusion; the other 359 were rejected. The resulting new database (EAT 3) contains more than 5,450 entries on almost 600 chemicals. It provides the most comprehensive published compilation of highly reliable ecotoxicity data covering the period 1970 to 2000 (ECETOC, 2003). The EAT 3 database is available as an Excel spreadsheet. For each entry there are 32 fields of information on the substance, test species, test conditions, test description, endpoint, results and source references. All the references are held at the ECETOC Secretariat in Brussels. An additional database consisting of ecotoxicity data from 'test kits' such as Microtox has been prepared and is available as a stand-alone database (EAT 4) or combined with EAT 3 (EAT 5). This report provides some examples of the use of the EAT 3 database, including comparisons between species, environments, acute and chronic exposures, and different life stages.

With careful consideration of the particular ecosystem for which protection is required, typical 'standard' species can be used as effective surrogates for other species within their larger taxonomic grouping (fish, invertebrates, algae). There seems to be a good possibility of replacing fish tests with tests using invertebrates, algae or tissue cultures. While this may prove satisfactory for the needs of the 'registration and evaluation' steps in the currently proposed regulation on the registration, evaluation, authorisation of chemicals in Europe (EC, 2003), the ecological approach laid down in the Water Framework Directive (EC, 2000) may require a reassessment of these conclusions.

Broad equivalence of sensitivity to narcotic chemicals has been demonstrated between higher taxa from freshwater and saltwater environments. However, the marine environment contains many aquatic taxa that are not represented in freshwater. Given the greater diversity of species present in marine waters compared to fresh waters, it is uncertain whether the current approach to freshwater effects assessment will be equally protective of saltwater species. There are, for example, no data on important marine taxa such as echinodermata, ctenophora and cephalopoda. Uncertainty as to the sensitivity of species from these groups has led to proposals that a marine predicted no-effect concentration (PNEC) should be derived, using larger application factors than those used for freshwater. Research should be encouraged that will generate data to provide a

scientific basis to answer the question whether or not an additional safety factor needs to be applied to protect saltwater ecosystems, and if so, what the magnitude of any such factor should be (ECETOC, 2003).

With regard to the relative sensitivity of life-stages, the sensitivity of the whole life-cycle is generally greater than its constituent stages, but for fish it is apparent that juvenile stages exhibit typically the greatest sensitivity (ECETOC, 2003).

A valuable aspect of the EAT 3 database is its improved capacity to examine the extrapolation from acute ecotoxicity data, to levels which are safe after chronic exposure. For more than half of the situations examined, an 'acute to chronic ratio' of less than 10 is evident. For the vast majority of situations the value 70, compared to the current value of 100, is more than adequate, providing a generous margin within the total factor (acute to ecosystem) of 1,000 to allow for extrapolations between the results of chronic studies and safe levels for ecosystems (ECETOC, 2003).

The EAT database relating to the effects of chemicals on marine and estuarine organisms is comparatively limited, especially for organic compounds. Although it is desirable that this database is extended for such species, the data reviewed and current marine risk assessment practice suggest a reasonable correlation between the ecotoxicological responses of freshwater and saltwater biota, at least for the classical aquatic taxa (i.e. fish, crustacea, algae). There does not appear to be any marked difference in sensitivity between freshwater and saltwater biota that systematically applies across all three trophic levels considered. Where evaluated, differences between trophic levels within each medium were generally equally significant (ECETOC, 2001). Such variation is implicitly covered by the use of assessment factors in current risk assessment practice. Overall, the use of freshwater acute effects data in lieu of, or in addition to, saltwater effects data for risk assessment purposes is not contradicted by the empirical data on cyanides reviewed in this report. The use of pooled freshwater and seawater data is therefore recommended. Under the circumstances, PNEC values have been be derived from the most sensitive result regardless of the medium.

6.2.1 Short-term effects

Fish

Reliable 96-hour LC_{50} values for cyanides are available for 19 fish species (Table 26).

Most tests have been conducted with fathead minnow (*Pimephales promelas*), brook trout (*Salvelinus fontinalis*), bluegill sunfish (*Lepomis macrochirus*), rainbow trout (*Oncorhynchus*

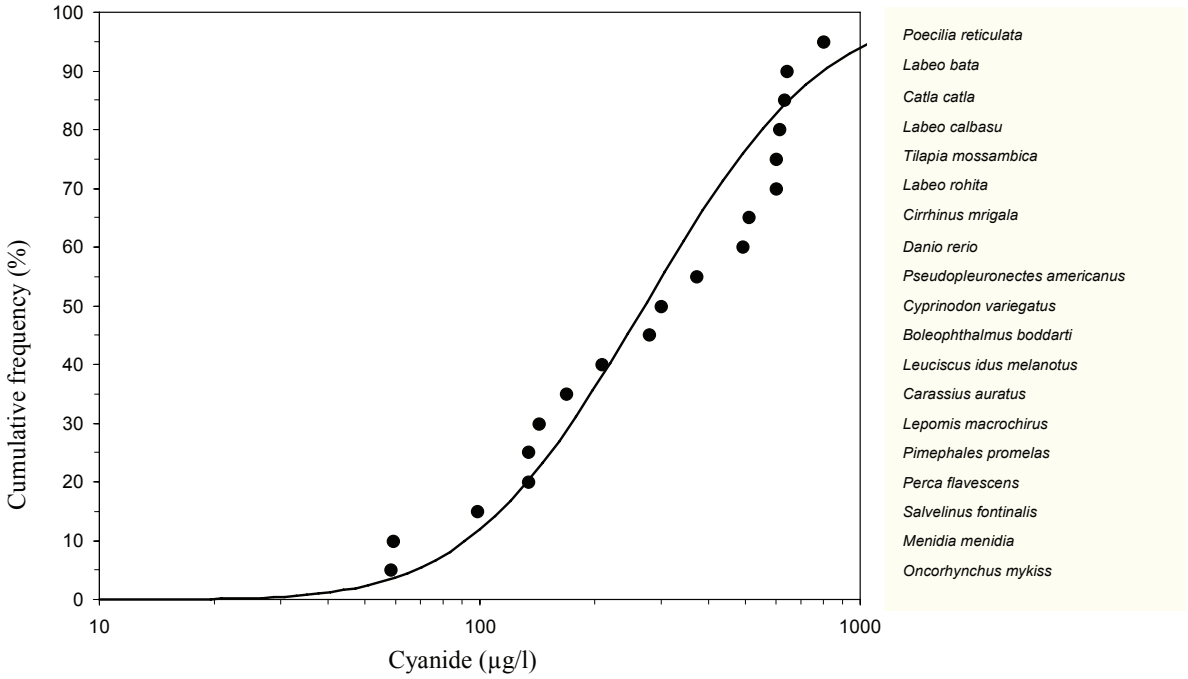
mykiss, formerly named *Salmo gairdneri*) and perch (*Perca flavescens*). Other taxa have only been tested once or within one study at different temperatures. For species tested several times, the minimum and maximum LC_{50} values can vary by up to one order of magnitude, which is explained by differences in test conditions and life stages tested. For cold water fish, sensitivity is usually higher at lower temperature (Section 6.2.3) and eggs and fry are usually less sensitive then juveniles. The cumulative plot of the species ranked by geometric mean for each species (Figure 16) reveals deviations from the normal distribution especially at the upper part of the distribution, starting with *Danio rerio*. These taxa have been tested under static conditions (or information about the type of exposure was not available) and the actual cyanide concentrations in the test vessels were probably lower than the reported nominal values.

Table 26: 96-hour LC_{50} values for fish

Name	Family	Number of LC_{50} values	LC_{50} (µg CN^-/l)	
			Mean [a]	Range
Freshwater				
Oncorhynchus mykiss	Salmonidae	15	57	27 - 97
Salvelinus fontinalis	Salmonidae	24	99	51 - 499
Perca flavescens	Percidae	9	134	73 - 325
Pimephales promelas	Cyprinidae	35	137	79 - 339
Lepomis macrochirus	Centrarchidae	22	144	72 - 351
Carassius auratus	Cyprinidae	1	169	
Leuciscus idus melanotus	Cyprinidae	1	209	
Danio rerio	Cyprinidae	1	490	
Cirrhinus mrigala	Cyprinidae	3	511	197 - 839
Labeo rohita	Cyprinidae	3	600	199 - 1,046
Tilapia mossambica	Cichlidae	3	600	194 - 1,068
Labeo calbasu	Cyprinidae	3	615	215 - 1,052
Catla catla	Cyprinidae	3	633	295 - 935
Labeo bata	Cyprinidae	3	639	250 - 1,046
Poecilia reticulata	Poeciliidae	1	800	
Saltwater				
Menidia menidia	Atherinidae	1	59	
Boleophthalmus boddarti	Gobiidae	1	279	
Cyprinodon variegatus	Cyprinidae	1	300	
Pseudopleuronectes americanus	Pleuronectidae	1	372	

[a] Geometric mean

Figure 16: Cumulative frequency distribution of 96-hour LC_{50} values[a] for fish



[a] Geometric mean

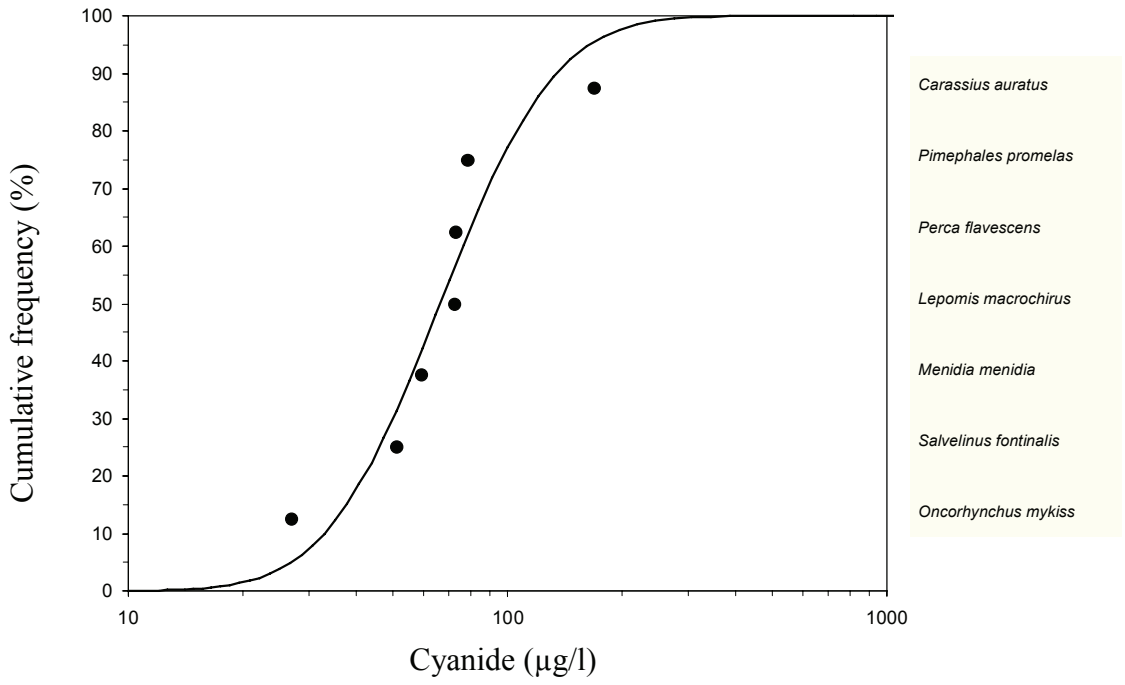
For a conservative analysis, the selection of tests was restricted to flow-through conditions and only the lowest LC_{50} per species was used (Table 27), resulting in a refined SSD curve (Figure 17). The lowest LC_{50} values were reported for salmonids, followed by the Atlantic silverside (*Menidia menidia*), bluegill sunfish (*Lepomis macrochirus*), perch (*Perca flavescens*) and cyprinids. The refined SSD is closer to log-normal and the estimated HC_5 of 26 µg/l, which predicts that for 95% of species the LC_{50} is higher than this value, corresponds to the lowest LC_{50} of 27 µg/l reported for rainbow trout (*Oncorhynchus mykiss*). This lowest reported LC_{50} was derived from a test with a water temperature at 6°C (Kovacs and Leduc, 1982a).

Table 27: 96-hour LC_{50} values[a] for fish under flow-through conditions

Name	Family	Number of LC_{50} values	LC_{50} (µg CN^-/l)
Freshwater			
Oncorhynchus mykiss	Salmonidae	7	27 - 73
Salvelinus fontinalis	Salmonidae	24	51 - 499
Lepomis macrochirus	Centrarchidae	14	72 - 351
Perca flavescens	Percidae	9	73 - 325
Pimephales promelas	Cyprinidae	33	79 - 339
Carassius auratus	Cyprinidae	1	169
Saltwater			
Menidia menidia	Atherinopsidae	1	59

[a] Geometric mean

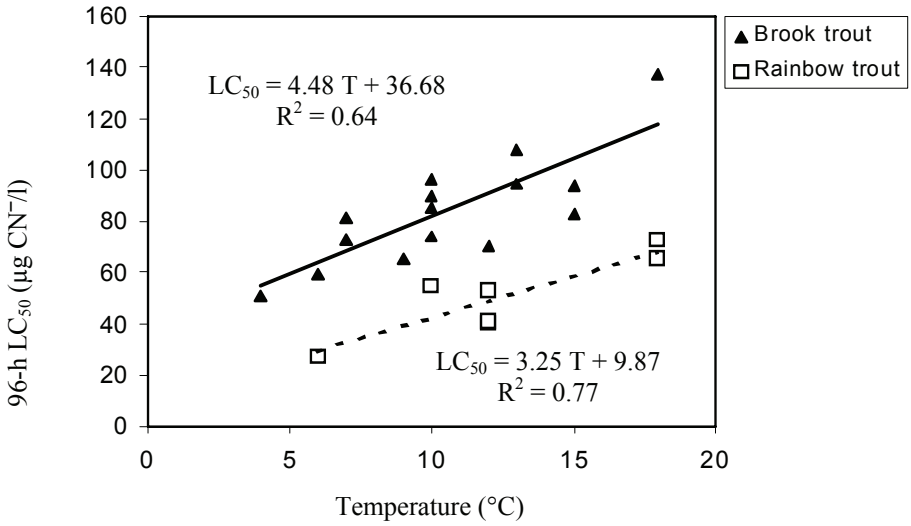
Figure 17: Cumulative frequency distribution of minimum 96-hour LC_{50} values for fish under flow-through conditions



A plot of LC_{50} values for two species of trout tested at different temperatures shows that rainbow trout (*O. mykiss*) are usually more sensitive than brook trout (*S. fontinalis*), and that for both

species sensitivity is closely related to water temperature (Figure 18). Thus, rainbow trout in 6°C cold water seems to describe a worst case scenario for acute risk of cyanides to fish. In the same experiment providing this low LC_{50} for rainbow trout (Kovacs and Leduc, 1982a), no mortality of fish was observed up to 17 µg CN^-/l. Effects on temperature and other environmental factors are discussed below in Section 6.2.3.

Figure 18: Effect of temperature on 96-hour LC_{50} values[a] of juvenile trout under flow-through conditions



[a] Geometric mean

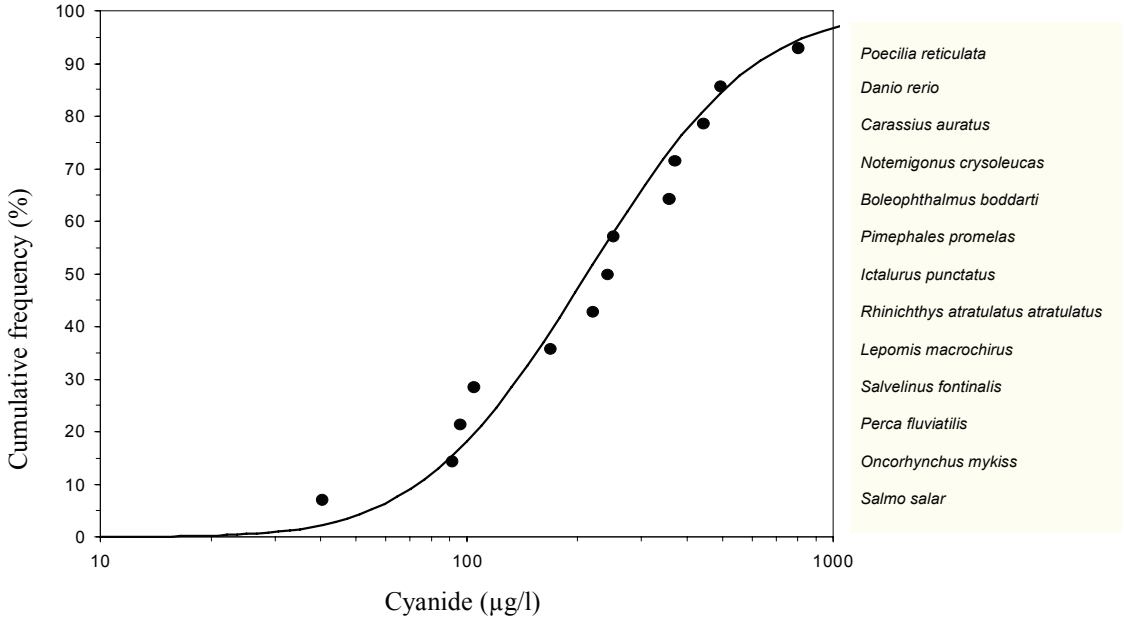
For some species, LC_{50} values were only available for shorter exposure times (Table 28, Figure 19). In principle, these 24-hour LC_{50} values are similar to those obtained for 96 hours. The similarity of the 24- and 96-hour LC_{50} values indicates that the onset of lethal effects of cyanides was very fast. Most of the lethal effects were usually observed within the first 24 hours of exposure. The lowest LC_{50} of 23 µg/l was reported by Alabaster *et al* (1983) for a salmonid (*Salmo salar*) at low oxygen levels (3.5 mg/l). At high oxygen levels (10 mg/l), which might be more realistic for salmon habitats, the 24-hour LC_{50} increased to 70 µg CN^-/l. The HC_5 for the 24-hour values was calculated to be 43 µg/l (95% confidence interval 19 - 71 µg/l).

Table 28: 24-hour LC_{50} values for freshwater fish

Name	Family	Number of LC_{50} values	LC_{50} (µg CN^-/l)	
			Mean [a]	Range
Salmo salar	*Salmonidae*	2	40	23 - 70
Oncorhynchus mykiss	*Salmonidae*	4	91	86 - 98
Perca fluviatilis	*Percidae*	1	96	
Salvelinus fontinalis	*Salmonidae*	1	104	
Lepomis macrochirus	*Centrarchidae*	6	168	79 - 280
Rhinichthys atratulatus	*Cyprinidae*	1	220	
Ictalurus punctatus	*Ictaluridae*	3	242	200 - 310
Pimephales promelas	*Cyprinidae*	1	250	
Boleophthalmus boddaerti	*Gobiidae*	1	356	
Notemigonus crysoleucas	*Cyprinidae*	3	369	300 - 540
Carassius auratus	*Cyprinidae*	5	441	175 - 3,250
Danio rerio	*Cyprinidae*	1	490	
Poecilia reticulata	*Poeciliidae*	1	800	

[a] Geometric mean

Figure 19: Cumulative frequency distribution of 24-hour LC_{50} values [a] for fish



[a] Geometric mean

In all, the lowest relevant acute LC_{50} values are around 25 µg CN^-/l for salmonids (rainbow trout and salmon) under worst-case conditions (low temperature or low oxygen level). Under other conditions or for other species (including other salmonids), LC_{50} values were generally above 50 µg CN^-/l.

For saltwater fish, the lowest LC_{50} was reported for the Atlantic silverside (*Menidia menidia)* with 59 µg/l (Brix *et al*, 2000), which is close to the geometric mean of the LC_{50} for rainbow trout.

The dose-response curve for lethal effects on fish is usually very steep, with concentrations without mortality (LC_0) or up to 10% mortality (LC_{10}) close to the LC_{50}, especially for the sensitive rainbow trout (Table 29). These data indicate that a factor of 5 applied to an LC_{50} should predict a safe concentration for survival.

Table 29: Comparison of 96-hour LC_0 (or LC_{10}) values[a] with LC_{50} values in freshwater fish

LC_0 (µg CN^-/l)	LC_{50} (µg CN^-/l)	Ratio $LC_{50}/LC_{0,10}$	Temperature (°C)	Reference
Lepomis macrochirus				
24	129	5.4	22	Monsanto, 1981a
Oncorhynchus mykiss				
17	27.0	1.6	6	Kovacs and Leduc, 1982a
31	40.5	1.3	12	Kovacs and Leduc, 1982a
58	65.5	1.1	18	Kovacs and Leduc, 1982a
24	67	2.8	12	Monsanto, 1981b
60	97	1.6	21	Skibba, 1981
82[b]	97	1.2	20	Tscheu-Schlüter and Skibba, 1986

[a] Geometric mean

[b] LC_{10}

Invertebrates

Records with effect data for invertebrates such as EC_{50} (e.g. *Daphnia* immobilisation) or LC_{50} values were available for 39 taxa. Test guidelines only exist for a few aquatic invertebrate species and test duration can vary depending on the species. In a first step to construct the SSD, a large number of taxa including all tests over 24 and up to 96 hours were considered and the geometric mean for each species was used (Table 30, Figure 20).

Table 30: Acute effects on invertebrates – LC_{50} or EC_{50} values

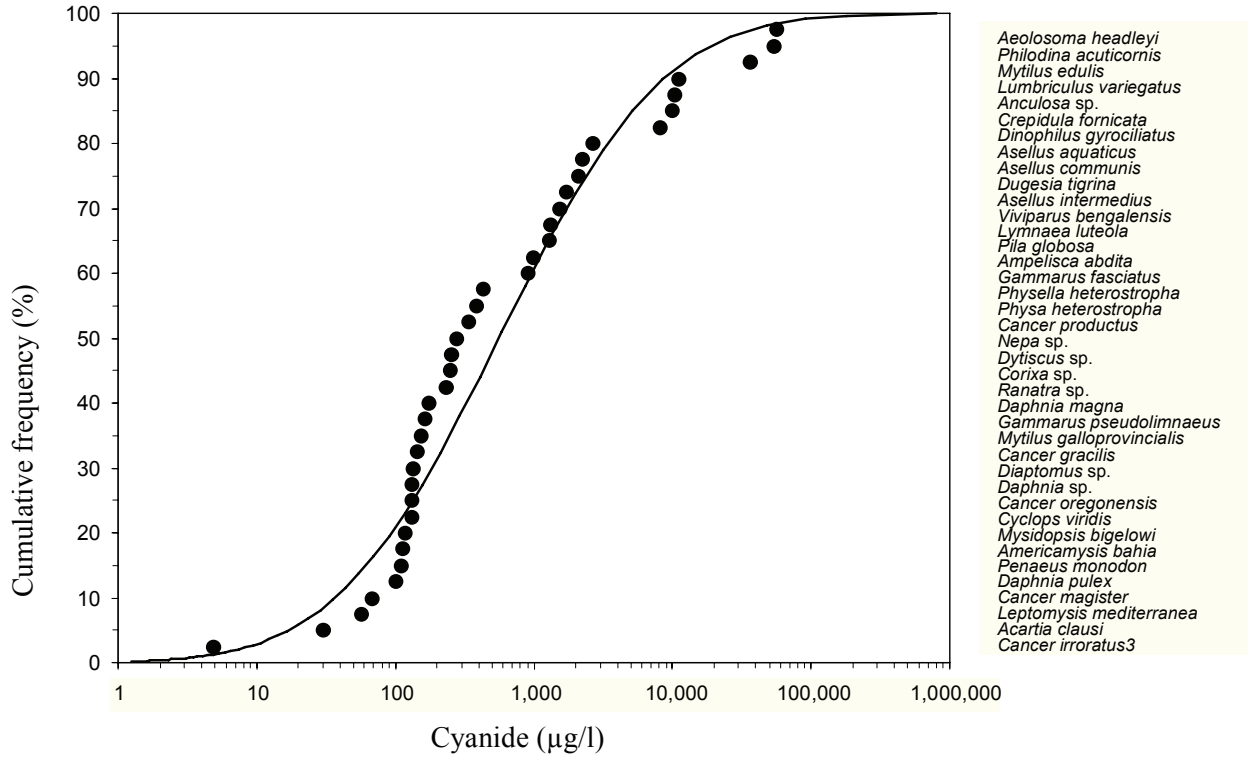
Scientific name	Group	Number of tests	LC_{50} or EC_{50} (µg CN^-/l)	
			Mean [a]	Range
Freshwater				
Daphnia pulex	*Crustacea*	11	100	1 - 420
Cyclops viridis	*Crustacea*	3	130	78 - 169
Daphnia sp.	*Crustacea*	3	132	80 - 173
Diaptomus sp.	*Crustacea*	3	133	82 - 173
Gammarus pseudolimnaeus	*Crustacea*	1	163	
Daphnia magna	*Crustacea*	6	174	40 - 1,900
Ranatra sp.	*Insecta*	3	230	228 - 232
Corixa sp.	*Insecta*	3	250	247 - 252
Dytiscus sp.	*Insecta*	3	252	246 - 259
Nepa sp.	*Insecta*	3	274	242 - 294
Physa heterostropha	*Mollusca*	3	383	272 - 740
Physella heterostropha	*Mollusca*	1	432	
Gammarus fasciatus	*Crustacea*	1	900	
Pila globosa	*Mollusca*	3	1,293	892 - 1,572
Lymnaea luteola	*Mollusca*	3	1,326	1,316 - 1,344
Viviparus bengalensis	*Mollusca*	3	1,550	1,532 - 1,577
Asellus intermedius	*Crustacea*	1	1,700	
Dugesia tigrina	*Turbellaria*	1	2,100	
Asellus communis	*Crustacea*	1	2,210	
Asellus aquaticus	*Crustacea*	1	2,680	
Anculosa sp.	*Mollusca*	10	10,402	7,000 - 14,000
Lumbriculus variegatus	*Oligochaeta*	1	11,000	
Philodina acuticornis	*Rotatoria*	1	54,000	
Aeolosoma headleyi	*Oligochaeta*	9	56,470	9,000 - 160,000
Saltwater				
Cancer irroratus	*Crustacea*	2	5	4 - 6
Acartia clausi	*Crustacea*	1	30	
Leptomysis mediterranea	*Crustacea*	2	57	37 - 88
Cancer magister	*Crustacea*	2	68	51 - 92
Penaeus monodon	*Crustacea*	1	110	

Table 30: Acute effects on invertebrates - LC_{50}/EC_{50} values (cont'd)

Scientific name	Group	Number of tests	LC_{50}/EC_{50} (µg CN^-/l)	
			Mean [a]	Range
Saltwater (cont'd)				
Americamysis bahia	*Crustacea*	1	113	
Mysidopsis bigelowi	*Crustacea*	1	118	
Cancer oregonensis	*Crustacea*	2	131	111 - 154
Cancer gracilis	*Crustacea*	2	144	135 - 153
Mytilus galloprovincialis	*Mollusca*	1	154	
Cancer productus	*Crustacea*	2	338	332 - 345
Ampelisca abdita	*Crustacea*	1	996	
Dinophilus gyrociliatus	*Annelida*	8	8,074	5,937 - 11,446
Crepidula fornicata	*Mollusca*	1	10,000	
Mytilus edulis	*Mollusca*	1	36,000	

[a] Geometric mean

Figure 20: Species sensitivity distribution for acute effects[a] on invertebrates

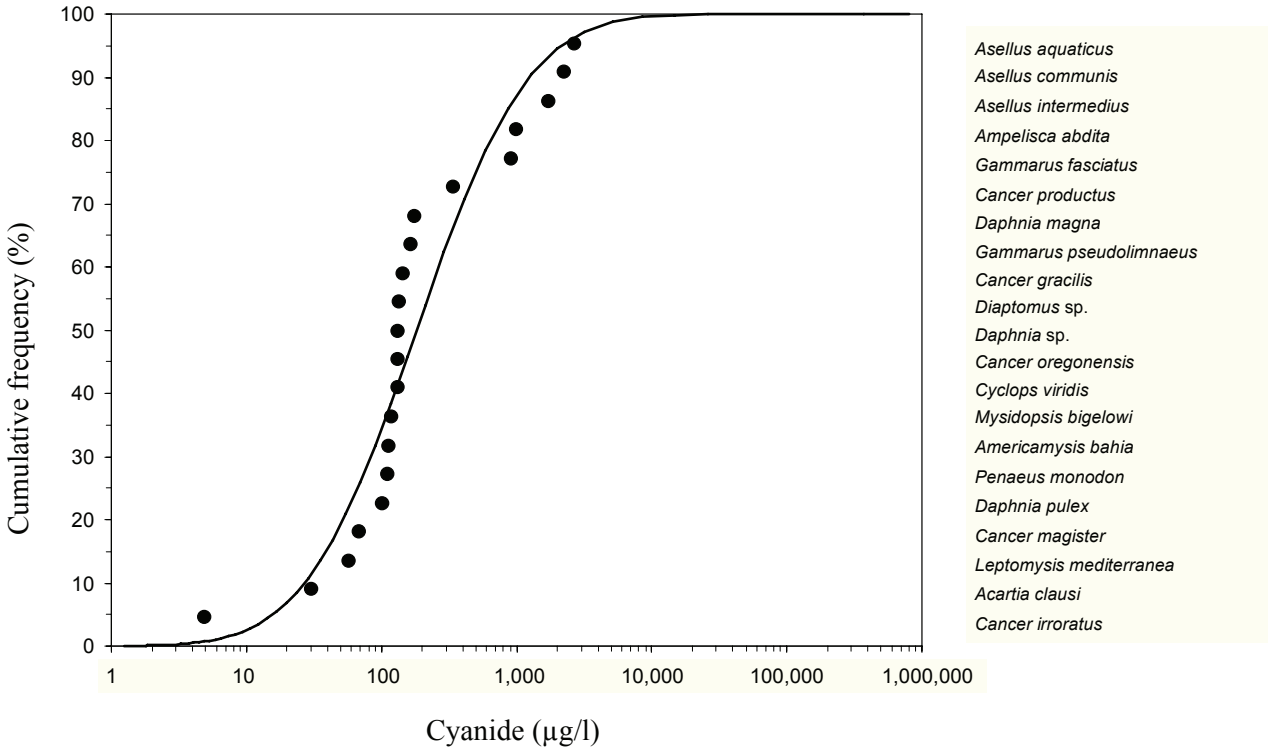


[a] Geometric mean of all LC_{50} or EC_{50} values per species

With one exception (48-hour LC_{50} of 154 µg/l for veliger larvae of the mollusc *Mytilus galloprovincialis* [Pavicic and Pihlar, 1982]), all taxa with mean values below 200 µg/l are crustaceans. Also, almost all maximum LC_{50}/EC_{50} values are lower than 200 µg CN^-/l, except for the variable EC_{50} values of *Daphnia pulex* (1 - 420 µg/l). Insects and crustaceans dominate the range between 200 and 1,000 µg/l. Again, a daphnid (*D. magna*) shows a large variability (40 - 1,900 µg/l). Insensitive taxa with LC_{50}/EC_{50} values above 1,000 µg/l are most of the molluscs, all tested oligochaeta, annelida, turbellaria and rotifera, but also isopoda (crustacea) of the genus *Asellus*. The data in the SSD diagram show some steps that indicate that the analysis should be split up for some groups. Therefore, the records for the 21 crustacean species representing the most significant group were analysed in more detail. The crustaceans were not split with respect to fresh- and saltwater species, because decapoda seem to be a sensitive group and were not tested in freshwater.

The SSD for crustaceans alone resulted in an HC_5 of 15 µg/l with a 90% confidence interval of 5 to 31 µg CN^-/l (Figure 21).

Figure 21: Species sensitivity distribution for acute effects[a] on crustaceans

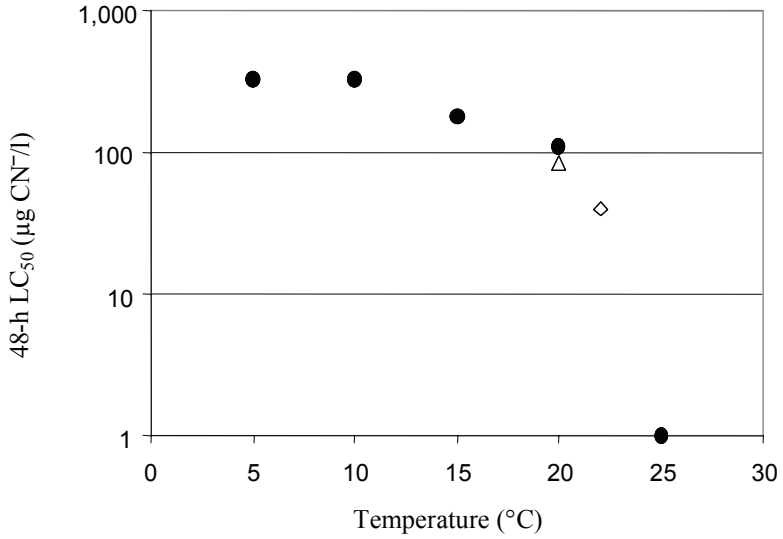


[a] Geometric mean of all LC_{50} or EC_{50} values per species

The lowest LC_{50}/EC_{50} values of 1 (48 h) and 3 µg CN^-/l (24 h) were reported for *Daphnia pulex* at a water temperature of 25°C (Cairns *et al*, 1978). All other values for *Daphnia* are higher than or equal to 40 µg CN^-/l. Cairns *et al* (1978) investigated the effect of water temperature on sensitivity of *D. pulex* and other animals. At 20°C they obtained a 48-hour LC_{50} of 110 µg/l, which corresponds well to the LC_{50} reported by Lee (1976 cited by Cairns *et al*, 1978) (Figure 22). Thus, the value for 25°C seems to be an outlier and the lowest reliable LC_{50} for *Daphnia* is 39.8 µg/l (Monsanto, 1981c).

The other species with an LC_{50} below 10 µg/l is the crab *Cancer irroratus*. This value was used to derive US-EPA national marine water quality criteria (cited by Brix *et al*, 2000). Brix *et al* (2000) conducted tests with four other species of *Cancer* that were on average 24 times less sensitive than *C. irroratus* (96-h LC_{50} 51 - 219 µg/l). Thus, the LC_{50} for *C. irroratus* seems to be overly protective. Except for the two discussed studies, all other LC_{50} values for crustaceans (and other invertebrates) are above 30 µg/l.

Figure 22: Effect of temperature on 48-hour LC_{50} values for Daphnia pulex [a] (Cairns *et al*, 1978)



[a] LC_{50} values for Δ *D. pulex* (Lee, 1976) and ◊ *D. magna* (Monsanto, 1981c) are shown for comparison

[b] For the value of Lee (1976), water temperature was not available and the standard of 20°C was assumed

Only three threshold values for survival of invertebrates could be found. For *Daphnia magna*, Monsanto (1981c) reported no mortality up to 23.3 µg CN^-/l (EC_{50} 39.8 µg/l), while Gillar (1962) found no mortality up to 100 µg/l (no LC_{50} available). All specimens of *Mysidopsis bigelow* survived for 96 hours at concentrations of up to 50 µg CN^-/l; LC_{50} values were around 100 µg/l (Lussier *et al*, 1985). These data for invertebrates also indicate that threshold concentrations for lethal effects of cyanides are close to the median effect levels.

Primary producers (algae and macrophytes)

According to ECB (2003), EC_{50} values from algae growth inhibition tests can be treated as short-term effects, while NOECs from these tests can be used for long-term effect assessment. Only a few studies provide EC_{50} values for effects of cyanide on algae or macrophytes (Table 31). Some tests lasted several days and none of them seems to have been conducted to current or Good Laboratory Practice (GLP) guidelines. Exposure was static in all tests.

Table 31: EC_{50} values for algae and macrophytes

Scientific name	Group	Time	EC_{50} (µg CN^-/l)	Temperature (°C)	Remark	Reference
Freshwater						
Chlorococcales sp.	*Chlorophyta*	24 h	45	20	Oxygen production	Krebs, 1991
Ankistrodesmus falcatus	*Chlorophyta*	10 d	1,250	25		Tscheu-Schlüter and Skibba, 1986
Navicula seminulum	*Diatomeae*	96 h	258 - 588	22 - 30	Different temperatures, soft or hard water	Academy of Natural Sciences, 1960
Myriophyllum spicatum	*Macrophyta*	32 d	20,000 - 28,600	20	Different endpoints	Stanley, 1974
Saltwater						
Nitzschia closterium	*Diatomeae*	72 h	57	21		Pablo *et al*, 1997a

Most of the values are higher than 200 µg/l, but Krebs (1991) reported an EC_{50} of 45 µg/l for oxygen production of *Chlorococcales* (green algae) and Pablo *et al* (1997a) determined an EC_{50} of 57 µg/l for the saltwater diatom *Nitzschia closterium.*

The only available test with macrophytes is not a short-term test because it was conducted over 32 days. For growth of the macrophyte *Myriophyllum spicatum*, EC_{50} values between 20 and 30 mg/l (depending on the endpoint) were reported by Stanley (1974). These values were obtained under static exposure conditions and measured cyanide concentrations were not given. The data suggest that macrophytes are much less sensitive than algae.

6.2.2 Long-term effects

A total of 30 NOECs could be found; the study data are summarised in Table 32.

It is acknowledged that some of the tests are not really long-term tests (e. g. the growth tests for *Mytilus* larvae) but these NOECs for sublethal effects were included here to cover more species and because the life-stages tested might last only a few days. Some of the NOECs were excluded from further consideration (non-shaded in Table 32), either because no effects were observed at the highest test concentration tested (and a true NOEC could not be established), the method was not described, or the endpoint was not considered to be ecologically relevant. The last criterion was applied to an artificial semination test with eggs and sperm of the rainbow trout *O. mykiss* (Billard and Roubaud, 1985) with a NOEC orders of magnitude lower than all other values. The activity of the electric organ of two *Gnathonemus* species was significantly affected at 5 µg/l (Lewis *et al*, 1992), but this physiological endpoint is also not considered to be ecologically relevant.

Table 32: Various long-term (sublethal) effects (NOECs) on algae, bacteria, invertebrates and fish[a]

Group 1 Latin name	Group 2	Effect	NOEC (µg CN^-/l)	T[b] (°C)	Exposure type	Time	Remark	Reference
Freshwater								
Algae								
Chlamydomonas	*Chlorophyta*	Population	≥ 10	15	Static	10 d	No consistent effects on growth rate	Cairns *et al*, 1978
Scenedesmus quadricauda	*Chlorophyta*	Population	12	27	Static	8 d	3% deviation from control, by regression	Bringmann and Kühn, 1978b,c
Chlorococcales sp.	*Chlorophyta*	Physiology	24		Static	24 h	Given as EC_{10} for oxygen production	Krebs, 1991
Microcystis aeruginosa	*Cyanophyta*	Population	28	27	Static	8 d	Threshold concentration from regression corresponding to 3% deviation from control	Bringmann and Kühn, 1978b,c
Phytoplankton, unspecified		Population	< 100	24	Static	10 d	Visual inspection of chlorophyll *a* over time, inhibition within the first 4 d, later compensation by blue-greens. Diatoms most sensitive group, growth rate higher than in controls	Shehata *et al*, 1988
Anabaena flos aquae	*Cyanophyta*	Population	≥ 700	30	Static	10 d	American Public Health Association guideline	Shehata *et al*, 1988
Bacteria								
Not stated	-	Population	9.2		Not stated		Method not described, BOD_5, BOD_{20} and nitrification as endpoint	Shkodich, 1966
Cnidaria								
Hydra viridissima	-	Population	≥ 200	30	Static	6 d	Half-time 4.5 h	Rippon *et al*, 1992

Table 32: Various long-term (sublethal) effects (NOECs) on algae, bacteria, invertebrates and fish[a] (cont'd)

Group 1 Latin name	Group 2	Effect	NOEC (µg CN^-/l)	T[b] (°C)	Exposure type	Time	Remark	Reference
Crustacea								
Gammarus pseudolimnaeus	*Amphipoda*	Reproduction	3.9	18	Not stated	98 d	Competition with *Asellus*	Oseid and Smith, 1979
Moinodaphnia macleayi	*Cladocera*	Reproduction	5.8	30	Static	5 d	NOEC as measured after 24 h. Initial nominal concentration 20 µg CN^-/l, half-time 14.4 h	Rippon *et al*, 1992
Asellus communis	*Isopoda*	Growth	27.9	18	Not stated	112 d	Alone	Oseid and Smith, 1979
Fish								
Oncorhynchus mykiss	*Salmonidae*	Reproduction	0.04	10	Static	0.7 d	May be relevant effect or outlier. Artificial semination, 40 min exposure, 10 d incubation: ≈ 63% fertilisation in controls, 58% at NOEC, 44% at LOEC 0.4 µg CN^-/l (1 µg KCN/l). Unrealistic conditions.	Billard and Roubaud, 1985
Oncorhynchus mykiss	*Salmonidae*	Growth	< 4.8	6	Flow-through	20 d	Fat gain and swimming ability: no statistically significant difference. NOEC estimated from diagrams	Kovacs and Leduc, 1982b
Oncorhynchus mykiss	*Salmonidae*	Biochemistry	4.8	6	Flow-through	20 d	60% of fat gain of controls, not significant	Kovacs, 1979
Salvelinus fontinalis	*Salmonidae*	Reproduction	5.4		Flow-through	144 d	Eggs spawned per female, LOEC = 11.2, EC_{50} ~ 30 µg/l	Koenst *et al*, 1977
Pimephales promelas	*Cyprinidae*	Reproduction	12.3	25	Flow-through	256 d	Eggs per female	Lind *et al*, 1977

Table 32: Various long-term (sublethal) effects (NOECs) on algae, bacteria, invertebrates and fish [a] (cont'd)

Group 1 Latin name	Group 2	Effect	NOEC (µg CN^-/l)	T [b] (°C)	Exposure type	Time	Remark	Reference
Saltwater								
Algae								
Champia parvula	*Rhodophyta*	Growth	3.9	20 - 22	Not stated	14 d	MATC [c] (female growth, cystocarps) = 5.5. NOEC = MATC/√2	Steele and Thursby, 1983
Nitzschia closterium	*Diatomeae*	Population	10	21	Static	72 h	LOEC < 10 µg/l, but effect < 10%	Pablo *et al*, 1997a
Ankistrodesmus falcatus	*Chlorophyta*	Population	265	25	Static	10 d	EC_{10} used as NOEC according to (ECB, 2003)	Tscheu-Schlüter, 1983
Crustacea								
Americamysis bahia	*Mysidacea*	Reproduction	> 30.4	20 - 25	Flow-through	Not stated	MATC > 43. NOEC = MATC/√2	Lussier *et al*, 1985
Echinoidea								
Anthocidaris crassispina	-	Development	125	28	Static	12 h	Short-term "maximum ineffective concentration" reported, but no test applied	Kobayashi, 1971
Mollusca								
Mytilus galloprovincialis	*Bivalvia*	Growth	3.2	20	Static	48 h	NOEC ~ LOEC/2 according to ECB (2003). Primary shell length 15% reduced at 6.4 µg/l. Growth of veliger larvae 46% of controls at 11.6 µg/l.	Pavicic and Pihlar, 1982
Mytilus edulis	*Bivalvia*	Physiology	< 18	15	Flow-through	14 d	Growth ≈ 70% reduction, not statistically tested. Glycine uptake ≈ 10% effect, significant	Thompson, 1984

Table 32: Various long-term (sublethal) effects (NOECs) on algae, bacteria, invertebrates and fish[a] (cont'd)

Group 1 Latin name	Group 2	Effect	NOEC (µg CN^-/l)	T[b] (°C)	Exposure type	Time	Remark	Reference
***Mollusca* (cont'd)**								
Chlamys asperrimus	*Bivalvia*	Development	5	18	Static	48 h	Endpoint % abnormal larvae	Pablo *et al*, 1997b
Fish								
Oncorhynchus mykiss	*Salmonidae*	Growth	4.8	6	Flow-through	20 d	Dry weight; 60% of fat gain of controls not significant	Kovacs, 1979
Lepomis macrochirus	*Centrarchidae*	Reproduction	1	25	Flow-through	289 d	Extrapolated from LOEC = 5: no spawning at all test concentrations except 1 early spawning at highest of 8 treatment levels (5 and 8 in the 2 controls)	Kimball *et al*, 1978
Jordanella floridae	*Cyprinodontidae*	Reproduction	12.6	25	Flow-through	Not stated	Extrapolated from LOEC = 63 for mean number of eggs laid/female during 1 oestrous cycle, time to maturity, different effects on embryos	Cheng and Ruby, 1981
Gnathonemus tamandua	*Mormyridae*	Physiology	< 5	25	Flow-through	Not stated	Electric organ activity, ecologically not relevant	Lewis *et al*, 1992
Gnathonemus petersi	*Mormyridae*	Physiology	< 5	25	Flow-through	Not stated	Endpoint ecologically not relevant	Lewis *et al*, 1992
Cyprinodon variegatus	*Cyprinidae*	Growth	29	22.4	Not stated	28 d	Not checked	Schimmel, 1981a cited by US-EPA, 2003

[a] Shaded records were used to construct SSDs
[b] Temperature
[c] Maximum acceptable toxicant concentration

For some species, significant effects were reported at the lowest concentration tested (this makes this concentration the lowest-observed effect concentration, LOEC). If the effect at this concentration was between 10 and 20%, the NOEC was estimated to be half of this concentration according to ECB (2003). If the size of the effect was not given or larger than 20% it was decided to apply an assessment factor of 5 to this 'LOEC' to estimate the NOEC. This was done to avoid ignoring the information and using higher, less conservative NOECs. The assessment factor was set to 5 as a compromise between a factor of 10, which is often used to extrapolate from short-term LC_{50}/EC_{50} to long-term NOECs, and the factor of 2 for the extrapolation from an effect up to 20% (ECB, 2003).

Application of the above criteria and extrapolation methods resulted in NOECs for 16 species, 11 for freshwater and 5 for saltwater species (shaded entries in Table 32). All of the NOECs are above 3 µg/l, with exception of one NOEC value of 1 µg/l of Kimball *et al* (1978), obtained by extrapolation from the reported 'LOEC' of 5 µg CN^-/l, the lowest concentration tested, for spawning of the bluegill sunfish (*Lepomis macrochirus*). The test duration was 57 days (eggs to juveniles) or 289 days (adults). The water delivery system was similar to that described by Brungs and Mount (1970 cited by Kimball *et al*, 1978) and the toxicant dispensing system was similar to that of Mount and Warner (1965 cited by Kimball *et al*, 1978). Water supply of control and treatment groups provided 95% replacement in 12 hours. The water in each test vessel was analysed (3 ×/wk) for HCN, pH and dissolved oxygen. The analytical method for HCN was based on oxidation of cyanide with chloramine T to cyanogen chloride. The cyanogen chloride was reacted with pyridine and norphenazone (3-methyl-1-phenyl-2-pyrozolin-5-one) to form a coloured dye, and the concentration was determined by colorimetry. Exposure from the stage of fertilised eggs to the 57-days juvenile state of the bluegill showed highly fluctuating results. The NOEC was difficult to establish but was certainly higher than 10 µg/l. Water supply of control and treatment groups provided 95% replacement in 2.4 hours. Exposure of adults for 289 days hardly affected survival up to 50 µg CN^-/l. Spawning occurred 13 times in the control group of 10 females, but did not occur at 5 to 65.6 µg CN^-/l. One out of 6 females spawned at 80 µg CN^-/l, but the number of eggs was much lower than in the controls. The authors concluded that spawning in the bluegill was completely inhibited at a level of 5 µg CN^-/l.

The resulting database covers NOECs for 4 different groups of algae (chlorophyta, diatomea, rhodophyta and cyanophyta), crustaceans, molluscs and different families of fish. No reproduction test data were available for *Daphnia magna*, a commonly used standard test species, but there were data on two other crustaceans. Furthermore, NOECs were not available for insects, rotifers or macrophytes. According to the LC_{50}/EC_{50} values, these taxa are less sensitive than the taxa for which NOECs are available. Consequently, although ECB (2003) asks for 10 or more long-term NOECs for species from at least 8 different taxonomic groups, the Task Force believed there were sufficient data on species from different trophic levels to conduct a probabilistic

assessment since the most sensitive species were covered. Thus, the calculation of an SSD to estimate a concentration protective for 95% of the species seemed justified.

The SSD for the combination of freshwater and saltwater species resulted in a HC_5 of 1.1 µg/l (90% confidence interval from 0.4 to 2.3 µg/l) (Figure 23). Using freshwater data only (hence without the molluscs, the diatoms and the red algae), the HC_5 was calculated to be 1.4 µg/l (90% confidence interval 0.4 - 2.8 µg/l) (Figure 24). For both SSDs, different statistical tests of 'goodness of fit' revealed no significant deviations from the normal distribution. For the freshwater SSD, the extrapolated value for the bluegill *Lepomis macrochirus* seems to be too low compared with the distribution of the other species sensitivities.

Figure 23: Species sensitivity distribution of sublethal NOECs for freshwater and saltwater species

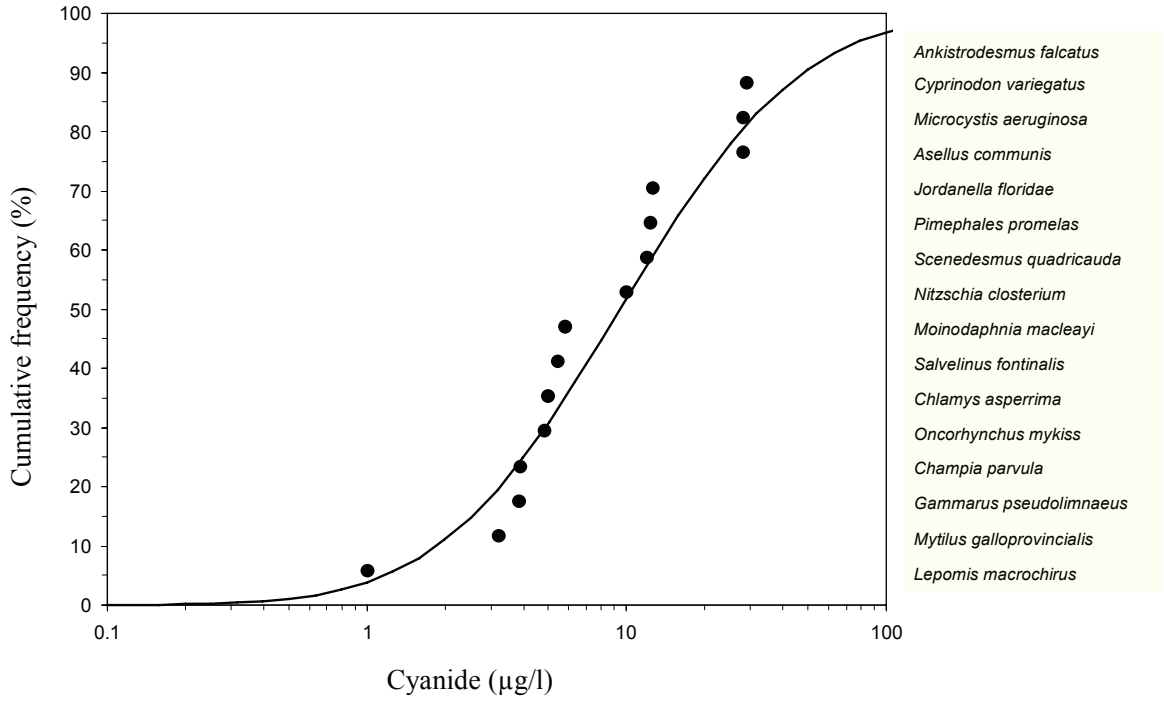
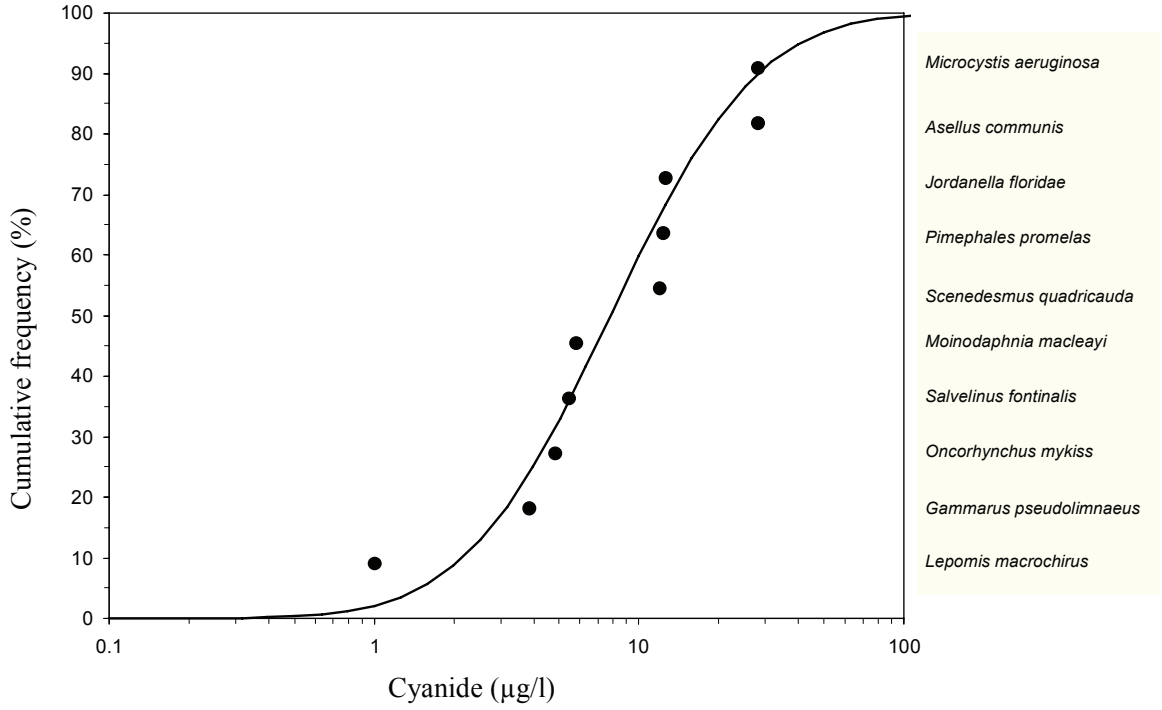


Figure 24: Species sensitivity distribution of sublethal NOECs for freshwater species



6.2.3 Factors affecting toxicity of cyanides to aquatic organisms

In fish, cyanide inhibits aerobic metabolism by irreversibly binding to the ferric ion in the haem moiety of cytochrome oxidase (Murgatroyd *et al*, 1998). If cyanide concentrations are high enough, fish are forced into anaerobic metabolism, and differences between species sensitivity can partly be explained by their different potential for anaerobic metabolism. Cyanide can also cause tissue damage and effects on the nervous system resulting in possible sublethal effects, e.g. on the swimming ability of fish and the reduction of metabolism (Chew and Ip, 1990; Murgatroyd *et al*, 1998). Observed effects on reproduction of fish might be explained by an increase of dopamine levels in the brain. This inhibits gonadotropin production, which reduces the reproduction of the fish (Szabo *et al*, 1991; Ruby *et al*, 1993a,b). Detoxification is mainly driven by the liver enzyme rhodanese, which converts cyanide to less toxic thiocyanate. This detoxification is more pronounced at high metabolic rates, which might explain why small fish, fish at high temperature or exercised fish are less sensitive (Chew and Ip, 1990; Murgatroyd *et al*, 1998). Due to low absorption, rapid volatilisation and rapid metabolism of cyanides, the potential for bioaccumulation of cyanides in fish is limited (Murgatroyd *et al*, 1998).

Herbert and Merkens (1952) noted that the sensitivity of rainbow trout (*O. mykiss*) increased with body size (length). Anderson and Weber (1975) found that, with increasing body weight (g) of the guppy (*Poecilia reticulata*), the 96-hour LC_{50} for KCN (in mg/l) increased according to $LC_{50} = 0.147\ (bw \times 10)^{0.72}$.

Nelson and Tolbert (1970) explained the low sensitivity of macrophytes compared to green algae by the presence of glycolate dehydrogenase found in the algae (*Chlamydomonas*, *Chlorella*, *Scenedesmus*, *Euglena* and *Acetabularia*). In contrast to glycolate oxidase, which is found in higher plants, glycolate dehydrogenase is cyanide sensitive.

In several studies, cyanide toxicity was investigated under different environmental conditions, especially temperature, oxygen level, pH and hardness.

Temperature

Sensitivity to cyanide was reported to increase with increasing water temperature in some studies while in other studies the opposite was observed. Increasing temperature increased the sensitivity of *Lepomis* fish and *Physa* snails, while no effect of temperature was found in the diatom *Navicula* (Academy of Natural Sciences 1960).

Cairns *et al* (1978) found no effects of temperature on the toxicity of cyanide to algae. Sensitivity of invertebrates increased with increasing temperature (but the oligochaete *Aeolosoma* showed a reverse response). This was explained by increased metabolic activity coupled with temperature potentiation of toxicant action on enzymes. At higher temperature, crustaceans produced more moultings, a more susceptible life stage. The authors observed a slight increase of sensitivity from 5 to 15°C in golden shiners (*Notemigonus crysoleucas*) and bluegills (*L. macrochirus*), and a more pronounced increase in goldfish (*Carassius auratus*) (5, 15, 30°C). Little influence of temperature was reported for channel catfish (*Ictalurus punctatus*) and rainbow trout (*O. mykiss*). In contrast to this, Kovacs and Leduc (1982a) found that rainbow trout were more sensitive to cyanides at 6 than at 12 or 18°C (LC_{50} of 27, 40, 65 µg CN^-/l). Smith *et al* (1978) found the same relation for the brook trout (*S. fontinalis*). LC_{50} values from different studies with these two species have been summarised above in Figure 18 (Section 6.2.1).

Chew and Ip (1990) reviewed the toxicity of cyanides to fish and concluded that "at slowly lethal concentrations, cyanide is more toxic at lower temperature whereas at rapidly lethal levels the reverse occurs; the reversal takes place at 0.1 mg/l HCN." This statement was originally made by Leduc (1984) who proposed that, at high HCN concentrations, the lethal effect is caused by disruption of vital organs such as the heart and the brain. At higher temperature, ventilation rate and metabolism of fish increase, resulting in a faster uptake of cyanide via the gills and a faster

distribution to the reaction sites. Leduc (1984) postulated that "at lower concentrations, death from HCN may better reflect the true metabolic conditions of the fish at different temperatures. Prolonged adaptation to cold water can induce (in fish) a higher oxygen consumption to meet energy needs and thus overcome the depressing effect of temperature on biochemical reactions. […] It would therefore appear that the increased toxicity of HCN at lower temperature would reflect the impact of cyanide on the much-needed oxidative pathways." Murgatroyd *et al* (1998) explained the typical decrease of toxicity to fish at higher temperature with higher metabolism of bioavailable cyanide by the enzyme rhodanese. They also note that higher temperatures also promote conversion of HCN to CN^-.

In one study investigating effects of temperature on sublethal effects in rainbow trout (*O. mykiss*), growth was more affected by cyanide at lower temperature (Kovacs and Leduc 1982a). Leduc (1984) explained the findings by inhibition of temperature compensation pathways at low temperature.

Dissolved oxygen

At low levels of dissolved oxygen, fish seem to be more sensitive. This is be explained by the higher uptake rate due to the increased ventilation and the direct effect of cyanide on the cytochrome oxidase. Alabaster *et al* (1983) reported a decrease of toxicity (24-h LC_{50} 71 to 21 µg CN^-/l) at low to high dissolved oxygen levels (3.5 - 10 mg/l) in smolts of the Atlantic salmon, *Salmo salar*. Smith *et al* (1978) also observed higher toxicity at lower dissolved oxygen levels for different species of fish. Downing (1954) reported that survival time of rainbow trout increases with oxygen level.

pH

The pH affects the cyanide speciation. Because HCN, as the most toxic form, predominates at low pH-values, toxicity decreases with increasing pH (Murgatroyd *et al*, 1998). Leduc (1984) added that "it is only at an abnormally high pH that the change in the proportion of the chemical species of cyanide could affect its toxicity."

Alkalinity and hardness

Water alkalinity and hardness seem to be unimportant for cyanide toxicity. The Academy of Natural Sciences (1960) investigated the effects of water hardness. Cyanide toxicity to fish (*Lepomis*), a snail (*Physa*) and a diatom (*Navicula*) was not affected by hardness of 10 or

100 mg $CaCO_3$/l. Cairns and Scheier (1963) found no effects of hardness (50 - 180 mg $CaCO_3$/l) on sensitivity of bluegills (*Lepomis*), while Kitamura (1990) found no effects of hardness on acute toxicity to *Tanichthys albonubes* (*Cyprinidae*).

6.3 Terrestrial organisms

6.3.1 Arthropods

Bond (1961c) studied the efficacy of HCN fumigation (8,000 mg HCN/m^3 in air; 7,120 ppm) in different insects (pests). The time to reach paralysis (of movement and respiration) in granary weevil (*Sitophilus granarius*) adults was 2 minutes, mealworm (*Tenebrio molitor*, darkling beetle) adults and larvae (mealworms) 15 minutes, desert locust (*Schistocerca gregaria*) adults 5 minutes, and black blow fly (*Phormia regina*) adults 1 minute, and the larvae 2 hours. While 15 minutes exposure was sufficient to kill *T. molitor* adults, the larvae continued to respire at a low rate (10%) after 4 hours of fumigation. *S. granarius* adults recovered within 2 hours, though respiration had completely ceased towards the end of the 15-minute exposure period. Reportedly, 7.1 ppm was the LC_{50} in *S. granarius* after 4 hours exposure. Another part of the work of Bond (1961a,b) addressed cyanide metabolism (detoxification) in *S. granarius and S. gregaria* (Section 4.3.4).

Repeated fumigation with HCN killed practically all larvae and eggs of the white citrus fly (*Aleurothrixus floccosus*) *in situ*. There was no difference in efficacy between three grades of HCN solution used to spray the orange trees (Del Rivero *et al*, 1974). Several insecticides and NaCN more or less inhibited the photomigration of dengue mosquito (*Aedes aegypti*) larvae. Second instar larvae were exposed to concentrations of 0.1, 1.0 and 10 mg NaCN/l in water (0.053, 0.53, 5.3 mg CN^-/l). While 0.053 and 0.53 mg/l did not have visible effects within 5 hours, 5.3 mg/l immobilised 50% of the larvae within 43 minutes (Burchfield and Storrs, 1954).

6.3.2 Vertebrates

Wiemeyer *et al* (1986) studied the acute oral toxicity of NaCN in 6 avian species. Groups of 3 to 20 male and female birds received 4 to 5 doses of NaCN in gelatine capsules to the proventriculus. The general symptoms observed were similar in all species and included slight coordination disturbance, rapid eye blinking, head-bowing and wing-droop followed by loss of coordination, convulsions and tail fanning, breathing disorders followed by death. Death occurred after between 15 and 30 min. Birds surviving 1 hour usually recovered. The LD_{50} values are summarised in Table 33. The three meat eating bird species (black vulture, American kestrel, eastern screech owl) had lower LD_{50} values and steeper dose-response curves than those feeding predominantly on plants.

Table 33: Acute oral toxicity of NaCN to birds

Species, sex, number per group	Test substance	LD_{50} (mg CN^-/kgbw)	Remark	Reference	CoR
Mallard duck (*Anas platyrhynchos*), 6 M, 6 F	NaCN	1.4		Henny *et al*, 1994	2e
Mallard duck, F (number not stated)	KCN	Not applicable [a]	Gavage. NOAEL based on biochemical parameters < 0.5 mg/kgbw/d	Pritsos and Ma, 1997	2e
Mallard duck (number and sex not stated)	NaCN	1.5	Bolus dose gelatine capsules	Clark *et al*, 1991	2e
Mallard duck, juvenile, 10 M/F	NaCN	18	Dosing over 5 days in drinking water	Stence *et al*, 1993a	1a
American kestrel (*Falco sparverius*), 5 M, 5 F	NaCN	2.1	Bolus dose gelatine capsules	Wiemeyer *et al*, 1986	2e
Black vulture (*Coragyps atratus*), 3 M, 3 F	NaCN	2.5	Bolus dose gelatine capsules	Wiemeyer *et al*, 1986	2e
Eastern screech owl (*Otus asio*), 5 M, 5 F	NaCN	4.6	Bolus dose gelatine capsules	Wiemeyer *et al*, 1986	2e
Japanese quail (*Coturnix japonica*), 5 M, 5 F	NaCN	5	Bolus dose gelatine capsules	Wiemeyer *et al*, 1986	2e
European starling (*Sturnus vulgaris*), 10 M, 10 F	NaCN	9	Bolus dose gelatine capsules	Wiemeyer *et al*, 1986	2e
Domestic chicken (*Gallus domesticus*), 3 F	NaCN	11.1	Bolus dose gelatine capsules	Wiemeyer *et al*, 1986	2e
Northern bobwhite quail (*Colinus virginianus*), juvenile, 10 M/F	NaCN	69	Dosing over 5 days in drinking water	Stence *et al*, 1993b	1a

[a] Animals were killed 2 hours after treatment

The impact of cyanide mining tailing ponds on deaths of wildlife in Arizona, California and Nevada between 1980 and 1989 was evaluated. In the three States an estimated 160 cyanide extraction gold mines were present at that time. Free cyanide concentrations in the tailing ponds were reported to be typically in the range of 200 to 300 mg CN^-/l, but some pond concentrations were as high as 700 or 9,000 mg/l. Wildlife deaths were reported to the respective State offices. There seemed to be episodes of high death rates at the mining sites, but a correlation to cyanide concentrations in tailing ponds was not made. A total of 6,997 deaths of birds, 519 mammals, 38 reptiles and 55 amphibians were reported between 1980 and 1989. The deaths were thought to be due to drinking water from the tailing ponds. The largest mammalian categories affected were bats and rodents. The numbers peaked in 1988. From 1986 to 1989, the number of mortalities per mine declined steadily while the number of mines increased. The authors expressed particular concern with regard to the impact of cyanide in tailings ponds to endangered species. They suspected that the reported numbers might only represent part of the true numbers due to inaccurate reporting of numbers and species identification. In addition, there were different requirements for reporting. For example, deaths were recorded only at mining sites, but not away from the mining sites, e.g. in case of migratory birds (Clark and Hothem, 1991; CoR 2e).

There were 9,512 cases of dead wildlife attributed to cyanide poisoning at Nevada gold mining operations between 1986 and 1991, 91% being birds (in particular waterfowl, shorebirds and perching birds), 7% mammals and 3% amphibians and reptiles (percentages stated by the authors). In order to derive lethal concentrations for migratory birds acute toxicity studies were conducted with overnight fasted adult (6 months old) Mallards (3/sex/group) that were given NaCN in gelatine capsules (0, 1, 2, 2.8, 4 and 5.7 mg/kgbw). The 24-hour LD_{50} was determined to be 2.7 mg/kgbw. The authors also studied exposure of wildlife animals at the tailing ponds. Waterfowl and shorebirds used the ponds for resting and hydration. The routes of exposure included inhalation of HCN, percutaneous absorption and ingestion during preening and sieving for invertebrates. Weak acid dissociable (WAD) cyanide was determined at different parts of 17 tailing ponds. At the discharge pipe the concentrations ranged from 8.4 to 216 mg/l (pH mostly between 9.3 and 11.3) in the interface between 23 and 132 mg/l (pH 7.2 to 10.5) and in the reclaim area between 7.1 and 86 mg/l (pH 7.8 to 11.3). Seven operators used detoxification procedures to reduce wildlife hazard. Cyanide concentrations usually decreased between the discharge and the reclaim area by a factor of 2 to 9. Another source of cyanide exposure were puddles of water collected on top of heaps of spoil in gold mining areas, with concentrations of 120 to 1,120 mg WAD CN^-/l. Dying birds were observed in the field at various concentrations of cyanide in the tailing ponds (as low as 16 mg/l WAD). The authors suggested that the absorption rate might be the critical factor for wildlife toxicity and that this factor might vary depending on the cyanide species (complexes) present. It was impossible to derive a safe concentration for wildlife from the data obtained in the study (Henny *et al*, 1994).

Pritsos and Ma (1997) tried to establish an acceptable level of cyanide in tailings ponds by studying the effect of sublethal doses of cyanide on biochemical parameters in young adult female Mallard ducks. Groups of ducks (number per group not stated) received 10 ml/kgbw of aqueous solutions containing 0, 0.5, 1 and 2 mg KCN/kgbw (0, 0.2, 0.4, 0.8 mg CN^-/kgbw or 0, 20, 40, 80 mg CN^-/l) by gavage. Two to 24 hours after administration of the test material the animals were killed. Blood samples were analysed for creatine kinase activity at 24 hours, mitochondrial respiration was studied after 2 hours in the mitochondrial fraction of heart, liver and brain homogenates, ATP levels were determined in frozen denatured tissues of heart, liver and brain by a luciferase-luciferin bioluminescence assay. A significant increase in blood creatine kinase levels was observed at dose levels from 0.4 mg CN^-/kgbw. Significant reductions in mitochondrial respiration rates and depletion of ATP were reported in all three tissues at the lowest dose tested. The authors concluded that sublethal concentrations of cyanide as low as 20 ppm could still pose a risk to birds. A NOAEL was not established.

A 5-day repeated exposure study according to OECD guideline 205 (OECD, 1984b) was performed in groups of 10 juvenile (7 days of age) Mallard ducks (*Anas platyrhynchos*) receiving nominal concentrations of 0 (3 groups), 100, 178, 316, 562 or 1,000 mg NaCN/l (0, 53, 94, 168, 298, 530 mg CN^-/l) in their drinking water. In an amendment to the report, the actual concentrations were given as 85.5 to 88.5, 152 to 158, 270 to 280, 481 or 855 mg $NaCN^-$/l, but measured concentrations (following re-analysis of the samples) were considerably lower (11.0 to 26.5% of nominal), both at day 1 and day 4, and averaged 21.2, 28.2, 39.6, 53.1 or 96.7 mg CN^-/l. Following the original report, after the 5-day exposure period the animals received untreated drinking water. All birds were from the same hatch, pen-reared and phenotypically the same as wild birds. The birds were immature and could not be differentiated by sex. The water was applied by a nipple water system. Body weights were determined at the beginning of the dosing period, on day 5 and at termination on day 8. Food and water consumption were recorded daily. No mortality was observed in the control or the 100 and 178 mg/l groups, while 4 of 10 animals died at 316 mg/l and all (10/10) animals at 562 and 1,000 mg/l died during the dosing period. Signs of toxicity included loss of coordination, lower limb weakness, lethargy and depression and were first noted on day 2 in the 316 mg/l group. Mortalities in this group occurred on day 2 and day 4 (2 each). In the surviving animals the symptoms had disappeared by the morning of day 6. In the two high-dose groups mortalities occurred on day 2 (3/10) and day 3 (7/10). Signs of toxicity included reduced reaction to external stimuli, depression, lethargy, wing drop, loss of righting reflex, prostrate posture and gasping. They were first noted in the afternoon of day 1. No clinical signs were observed in the 2 lower dose groups. A statistically significant dose related reduction in body-weight gain was observed in all dose groups. Birds dosed with 100 mg/l continued to show a reduction in body-weight gain during the observation period. The reduction in body-weight gain was accompanied by a marked dose-related reduction in feed and water consumption throughout the dosing and recovery periods. According to the authors, the birds receiving NaCN reduced their water consumption to a

point where many of the deaths may well have been due to dehydration rather than to treatment alone. Based on the original nominal concentrations, the 5-day LC_{50} was reportedly determined to be 340 mg NaCN/l (180 mg CN^-/l) or approximately 75 mg/kgbw using the average drinking water consumption (415 ml/kgbw) of the 316 mg NaCN/l group. The concentration at which no mortality occurred was 178 mg NaCN/l (94 mg CN^-/l) or a dose of 55 mg/kgbw using the average drinking water consumption of 583 ml/kgbw at 178 mg NaCN/l. The lowest-observed effect level (LOEL), based on effects on body weight, feed and water consumption, was 100 mg NaCN/l (53 mg CN^-/l) or 49 mg/kgbw using the average drinking water consumption of 916 ml/kgbw at that dose (Stence *et al*, 1993a). Although the authors based their original evaluation on the nominal concentrations, the analytical concentrations were considerably lower. Therefore, the Task Force considered it more appropriate to base the evaluation on the measured concentrations. The cyanide concentration at which no mortality occurred would be 28.2 mg/l or 16.4 mg/kgbw. The LC_{50} would correspond to approximately 43 mg/l or 17.8 mg/kgbw and the LOEL to 21.2 g/l or 19.4 mg/kgbw.

The same authors conducted a similar experiment, using the same study design according to OECD guideline 205, in juvenile northern bobwhite quails (*Colinus virginianus*) (10 days of age). The 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) in their drinking water for 5 consecutive days, followed by 3 days of observation. Analytical measurement of the test solutions confirmed the nominal concentrations with the exception of the 178 mg NaCN/l sample at day 5. That sample had only 9.14% of the nominal concentration. The evaluation was therefore based on the nominal concentrations. No mortality was observed in the controls or at 100, 178 and 316 mg/l, while 1 of 10 animals died at 562 mg/l and all (10/10) animals at 1,000 mg/l died during the dosing period. Signs of toxicity included wing drop and lethargy at 178 mg/l (days 3 to 7) and, additionally, depression and reduced reaction to external stimuli at 316 mg/l (1 animal, day 0 to 7). At 562 mg/l, a single mortality was noted on day 6 and symptoms observed from day 0 to day 7 included, in addition to the symptoms in other dose groups, loss of coordination. At 1,000 mg/l, mortalities occurred on days 0 and 1 (3 each), one each on day 2 and 3 and two on day 4. Signs of toxicity were observed from day 0. In the animals surviving animals the lower concentrations the symptoms had disappeared by the afternoon of day 7. A statistically significant dose-related reduction in body-weight gain was observed from 178 mg/l. Birds dosed with 100 mg/l did not show any effect on body-weight gain compared to the control groups. The reduction in body-weight gain at 178 to 1,000 mg/l was accompanied by a dose-related reduction in food and water consumption throughout the dosing and recovery periods. According to the authors, the birds receiving NaCN reduced their water consumption to a point where many of the deaths may well have been due to dehydration rather than to the treatment alone. Due to this fact, the intake of cyanide per kg body weight in the two high-dose groups was in the range of the intake of the mid dose groups. The 5-day LC_{50} was determined to be 705 mg NaCN/l (374 mg CN^-/l) or approximately 69 mg/kgbw using the average drinking water consumption (98 ml/kgbw) at

562 mg NaCN/l. The concentration at which no mortality occurred was 316 mg NaCN/l (168 mg CN^-/l) or 35 mg CN^-/kgbw using the average drinking water consumption of 210 ml/kgbw at 316 mg NaCN/l. The NOEL, based on effects on signs of toxicity, body weight, and water consumption, was 100 mg NaCN/l (53 mg CN^-/l) or 33 mg CN^-/kgbw based on the average drinking water consumption of 330 ml/kgbw at that dose (Stence *et al*, 1993b).

Following the observed deaths of wildlife animals in the neighbourhood of cyanide extraction gold mines (Clark and Hothem, 1991 above), the acute toxicity of NaCN to a number of species was determined. Mallard ducks (*Anas platyrhynchos*) were most sensitive with an LD_{50} of 2.9 mg/kgbw followed by little brown bats (*Myotis lucifugus*, LD_{50} 8.4 mg/kgbw), house mice (*Mus musculus*, LD_{50} 8.7 mg/kgbw) and white-footed mice (*Peromyscus leucopus*, LD_{50} 28 mg/kgbw). Slopes of the dose-response curves were reported to be extremely steep for Mallards and house mice. Little brown bats showed delayed mortality (Clark *et al*, 1991; CoR 4a).

Groups of 8 to 16 wild opossums, *Trichosurus vulpecula* (sex not stated), were dosed with solutions of NaCN by oral gavage. The median lethal dose (LD_{50}) was 8.86 mg NaCN/kgbw (4.5 mg CN^-/kgbw) (Bell, 1972; CoR 2e)

Sterner (1979; CoR 2e) studied the effects of NaCN to be used in applications as predacides for the control of coyotes (*Canis latrans*) to prevent them from attacking sheep. Oral application, to the back of the mouth of one wild trapped coyote per dose, of 4, 8, 16, 32, or 64 mg/kgbw resulted in death of the animals within 5 to 41 minutes from 8 mg/kgbw. The coyotes were immobilised within 1 to 13 minutes. The 4 mg/kgbw dose was sublethal.

Conclusion

With regard to acute toxicity to wildlife vertebrates, birds (in particular Mallards) seem to be the most sensitive species. The toxicity of cyanides will depend on the dose rate and the conditions of exposure. It is noteworthy that in the 5-day drinking-water study of Stence *et al* (1993a) the LC_{50} was about a factor of 10 higher than in the study of Clark *et al* (1991) who applied a bolus dose. The studies of Stence *et al* (1993a,b) are more representative of the situation that would occur close to tailing ponds containing cyanide. The steep dose-response curve and the relative quick recovery after sublethal doses of cyanide that were observed in the 5-day studies are in accordance with the results of studies in experimental animals. It confirms the predominance of acute toxic effects over possible effects after repeated exposure. Although a clear NOAEL could not be established in the existing studies the LOEL of 53 mg CN^-/l based on reduced food and water intake of Mallard ducks (Stence *et al*, 1993a) could be used as a starting point to derive a level that would probably prevent significant losses of wildlife. For the field situation, inhalation

and dermal exposure cannot be excluded. Uptake will also depend on the availability of alternative uncontaminated drinking water. All authors have, however, considered the oral uptake of contaminated water as the main source of cyanide exposure for birds. Thus a factor of 10 on the LOEL resulting in a concentration of about 5 mg CN^-/l may be a realistic estimate for a concentration that should protect wildlife living around cyanide sources.

Based upon the available data, avian species appear to be of comparable sensitivity to laboratory animals (Section 8.1 and 8.3).

6.3.3 Plants

Plants or their seeds and tubers may be exposed in the environment to cyanide via air and through soil and pore water. In addition, certain plants may themselves be sources of cyanide when cyanogenic glucosides are metabolised or when certain metabolic conditions prevail (Section 4.1.3 Biogenic Sources).

In green plants, in addition to inhibition of aerobic respiration, another mechanism of cyanide toxicity has been suggested: the inhibition of photosystem I. Experiments with chloroplasts and purified spinach plastocyanin indicated that cyanide inactivated plastocyanin by reaction with the central copper ion, thus interrupting the electron transport chain of the photosystem (Berg and Krogmann, 1975).

Seeds and tubers

Seeds of radish (*Raphanus sativus*) and spring rye (*Secale cereale*) were exposed to HCN in concentrations of 250 or 1,000 ppm (281 and 1,124 mg/m^3) for 1, 4, 15, 60, 240 and 960 minutes under dry and moist conditions. The exposure had no significant influence on germination success, but induced a marked delay in germination in a dose-related manner. While seeds exposed for 1 minutes (rye and radish) and 4 minutes (rye) at 1,000 ppm showed no difference from controls, seeds exposed for 15 minutes showed a delay of germination of 20 hours (radish) and 4 hours (rye), and 67 hours (radish) and 49 hours (rye) after 960 minutes exposure (Barton, 1940).

In other experiments when potato (*Solanum tuberosum*) tubers and seeds of several different plants were exposed to cyanide solutions, the exposure promoted germination of the seeds and sprouting of the tubers. Exposure to cyanide (as aqueous KCN solution) for 1 hour induced sprouting at a range of concentrations between 0.5 and 5.0 g/l (200 and 1,998 mg CN^-/l) and caused rotting of the tuber at 10 g/l (3,996 mg CN^-/l) (Denny, 1932), most likely due to toxicity.

Peeled, dormant apple (*Malus domestica*) seeds were exposed to 1 mmol HCN/l in air. Treatment for 6 hours broke the dormancy and stimulated germination and growth in a 12-hours light/dark cycle, but not in the dark. This treatment initially reduced oxygen consumption but did not inhibit cyanide-resistant respiration. It also induced β-cyanoalanine synthase activity. The authors discussed a specific effect of cyanide in the regulatory control of dormancy in apple seeds (Bogatek *et al*, 1991).

From a larger variety of seeds which had shown a similar effect, pigweed (*Amaranthus albus*) and lettuce (*Lactuca sativa*) were selected for germination experiments. KCN concentrations between 0.003 and 3 mmol/l (0.078 and 78 mg CN^-/l) increased germination in the dark of both seeds from approximately 20 to 30% in the untreated controls up to 60 to 80% of the seeds in the treated cultures. At 10 mmol/l (260 mg CN^-/l) germination success dropped to a rate near the control range, probably due to toxicity (Hendricks and Taylorson, 1972).

The germination success of seeds of two species of Leguminosae (*Rhynchosia minima* and *Clitoria ternatea*) incubated for 24 hours in different concentrations of NaCN solution was investigated. Concentrations up to 20 mg/l (10.6 mg CN^-/l) had no noticeable effect, while 50 mg/l to 500 mg/l (26.5 to 265 mg CN^-/l) stimulated germination, the maximum being at 100 mg/l (53 mg CN^-/l). At 1,000 mg/l (531 mg CN^-/l) germination was much lower and the seedlings displayed reduced growth while 2,500 mg/l (1,327 mg CN^-/l) inhibited germination almost completely. The stimulation of germination at 100 mg/l (53 mg CN^-/l) showed a maximum at 24 hours. Shorter treatment was less effective and so too was treatment for 48 hours and 72 hours, indicating an inhibitory, toxic effect (Mullick and Chatterji, 1967).

Corn (*Zea mays*) seeds were fumigated at 1, 2 or 3 lb HCN/1,000 ft^3 (14,200 ppm, 16,000 mg/m^3; 28,500 ppm, 32,000 mg/m^3; 42,800 ppm, 48,100 mg/m^3) for 72 hours. Germination success was reduced in moist seeds (15% water content), but was not affected in dry seeds (9% water content) (Strong and Lindgren, 1961). A slight, similar effect attributed to incomplete removal of HCN in moist seeds after fumigation was found in a comparable experiment in Sudan grass (*Sorghum vulgare*); other sorghum varieties showed no reduction in germination efficiency (Strong and Lindgren, 1963). In oats (*Avena sativa*), barley (*Hordeum vulgare*), wheat (*Triticum aestivum*), rice (*Oryza sativa*) and flax (*Linum usitatissimum*), HCN treatment did not affect germination success under similar conditions (Strong and Lindgren, 1959a,b,c,d, 1960).

Root exposure via soil or water

The possibility of using NaCN solutions to eradicate undesirable plants has been investigated in dandelion (*Taraxacum officinale*), plantain (*Plantago major*), crab grass (*Digitaria sanguinalis*), quack grass (*Agropyron repens*), foxtail (*Alopecurus pratensis*), European bindweed

(*Convolvulus arvensis*), fledge bindweed (*Convolvulus sepium*), honeysuckle (*Lonicera dioica*), privet (*Ligustrum vulgare*), gardenia (*Gardenia jasminoides*), and poison ivy (*Rhus toxicodendron*). Pouring 1 litre of 0.5 to 1% NaCN solutions (≈ 2,655 to 5,310 mg/m²) onto 1 m² of the soil or into holes adjacent to the plants left most plants more or less undamaged. Concentrations of 2% (10,620 mg/m²) were lethal to most species, and 3% (15,930 mg/m²) or higher killed all plant species investigated (McCool, 1945). Because the application procedure does not result in well defined concentrations in the test soil, the test concentrations are difficult to compare to those in other studies.

To study the suitability of willow (*Salix viminalis*) plants for the phytoremediation of cyanide-contaminated soils, the toxicity of KCN to saplings grown from cuttings of basket has been investigated. While the plants survived growing in a nutrient solution with 0.4 mg CN^-/l showing only a transient reduction in transpiration, 2, 8 and 20 mg CN^-/l reduced the transpiration significantly and killed the plants after 3 weeks (2 mg/l) or less than 1 week (8 and 20 mg/l). The EC_{50} (transpiration, 72 h) was determined to be 1.8 mg CN^-/l; the EC_{10} was 0.3 mg/l. In a similar experiment with willow saplings grown in sand irrigated with KCN solutions, the plants survived 20 mg CN^-/l, albeit showing a reduced transpiration throughout the entire test duration of 423.5 hours (17.6 d). A concentration of 50 mg CN^-/l was lethal within approximately 1 week under these conditions. In other experiments it was evident that the plants had a high potential to absorb and metabolise cyanide (Section 4.3.5 for details) (Trapp *et al*, 2001a,b; Larsen *et al*, 2004).

Roots of *Vicia faba* immersed in solutions of 50 mmol KCN/l (1,300 mg CN^-/l) showed an increase in anaphase aberrations, but only in the presence of oxygen (Lilly and Thoday, 1956).

Respiratory and photosynthetic rates of the first pair of attached leaves of 15 day old bean seedlings (*Phasaeolus vulgaris*) grown in nutrient solution with and without cyanide were measured. Cyanide (as KCN) at a concentration of 0.1 mmol/l (2.6 mg CN^-/l) inhibited growth, respiration and photosynthesis of the seedlings by 50, 45 and 34%, respectively. On a percentage basis, inhibition of growth, respiration and photosynthesis by cyanide was greater in the presence than in the absence of iron. Similar effects were found with wheat (*Triticum aestivum*) seedlings at a cyanide concentration of 0.5 mmol/l (13 mg CN^-/l) (Israelstam, 1968; Alam and Israelstam, 1975).

The potassium uptake rhythm of a flow-medium culture of duckweed (*Lemna gibba*) was investigated. NaCN at 0.1 or 0.3 mmol/l (2.6, 7.8 mg CN^-/l) reduced the average rate of potassium uptake, without markedly decreasing the amplitude of the rhythm, indicating a leaking of potassium from the cells. Addition of 1 mmol/l (26 mg CN^-/l) induced partial inhibition of the potassium uptake, while 3 mmol/l (78 mg CN^-/l) inhibited the uptake entirely (Kondo and Tsudzuki, 1980).

It is assumed that cyanogenic soil bacteria in the root zone of plants may inhibit the growth of plants under particular conditions. Potato plants growing in the presence of HCN-producing *Pseudomonas* species showed reduced growth compared with growth under non-cyanogenic conditions, but only if ferric iron was present at the same time (Bakker *et al*, 1991). A non-cyanogenic strain of the same bacterium had no effect.

Exposure of leaves or stem via air

Young plants of tomato (*Lycopersicon esculentum*), buckwheat (*Fagopyrum esculentum*) and tobacco (*Nicotiana tabacum*) were exposed to a continuous flow of air containing HCN at a concentration of 1,000 ppm (1,124 mg/m^3), tomato also at concentrations of 16, 63, 250 ppm (18, 71, or 281 mg/m^3) for periods of 1 to 960 minutes. Immediate damage to 50% of the leaf area was observed at 1,000 ppm after 4 to 12 minutes. Lethal damage to tomato plants was observed after exposure of plants to 16 ppm for 15 or 60 minutes, but not for longer or shorter treatment periods. The description of effects in the paper concentrated on the short-term effects visible immediately after treatment. The progress of lesions of treated plants in the days following treatment was only discussed briefly and not quantified (Thornton and Setterstrom, 1940).

Long thin stolons of strawberry begonia (or geranium) (*Saxifraga sarmentosa*) were treated with cyanide in solution and as a gas (equilibrium concentration above solutions) in the range of 0.1 to 5 mmol/l KCN (2.6 to 130 mg CN^-/l). Cyanide strongly inhibited the transport of ^{137}Cs and of natural ^{14}C assimilates. The effect was completely reversible within 24 hours. In conclusion, as judged by a variety of approaches, cyanide inhibited active pumping mechanisms in the sieve tubes. The gaseous form was much more effective (Qureshi and Spanner, 1973).

Phytotoxicity from HCN fumigation, as a potential disinfestation treatment, was determined in several varieties of Hawaiian cut flowers and foliage, including Zingiberaceae (ginger), Heliconia, Orchidaceae, Marantaceae, Lycopodiaceae, Agavaceae and Proteaceae. Concentrations tested were 2,500, 3,700, 4,600 and 5,500 ppm HCN (2,810, 4,159, 5,170 and 6,182 mg/m^3) for 30 minutes. Exposure to 2,500 ppm (2,810 mg/m^3) was safe for all plants and flowers except two types of Proteacea which were slightly damaged. Exposure to 3,700 ppm (4,159 mg/m^3) caused damage (discoloration) to ginger and two additional kinds of Proteacea (Hansen *et al*, 1991a,b).

The safety of fumigation of fruit trees with HCN at concentrations of up to 10,000 mg/m^3 (8,900 ppm) for 60 minutes was tested in field experiments using mobile cells with an air circulation system. Apricot trees (*Prunus armeniaca*) tolerated the fumigation without obvious damage. The leaves of young pear trees (*Pyrus communis*) were slightly damaged after exposure

to 4,000 mg/m^3 (3,560 ppm) for 15 minutes. Exposure to sunlight should be avoided during and after the exposure to HCN (Geier and Mathys, 1949).

Summary: plant toxicity

When roots are exposed to cyanide in soil pore water, macrophytes appear to be very resistant to cyanide in comparison to aquatic algae. Concentrations in excess of 1 mg/l seem to be tolerated in most cases. Dormant seeds and tubers show higher resistance if exposed by air or water. HCN has been successfully used at concentrations in excess of 1,000 ppm (1,124 mg/m^3) for the fumigation (elimination of parasitic insects) of different types of nutrient grain. Under conditions suitable for sprouting, exposure to cyanide may break the dormancy of seeds and tubers. When whole plants are exposed to HCN in air for short periods up to 60 minutes in order to eliminate parasitic insects, most species seem to tolerate exposures of up to several thousand ppm. A single paper (Thornton and Setterstrom, 1940) reported toxicity to young tomato plants at a concentration of 16 ppm for 15 to 60 minutes. In comparison to the other plant data this is an unexplainable finding. There are no laboratory data about effects of long-term exposure of plants to cyanide. The use of willows for phytoremediation indicates that the comparatively high resistance of plants to cyanide is maintained over extended time.

6.4 Ecosystems

Burning of biomass such as wood, grass and leaves can release dangerous amounts of cyanide that can poison water supplies. A study of wildfires in North Carolina found that nearby streams were contaminated by 49 µg/l of free cyanide, a level high enough to kill rainbow trout. The authors concluded that forest and brush fires might play a major role in fish kills (Barber *et al*, 2003).

6.5 Summary and evaluation

Micro-organisms show a wide range of sensitivity towards cyanide with toxicity thresholds between 0.001 mg/l and more than 1,000 mg/l in resistant strains. In addition to this wide range, adaptive responses have been demonstrated which may increase the toxicity threshold of cyanide in a particular organism by several orders of magnitude. The metabolic and respiratory systems may be adapted in the course of a few days or weeks. A study with soil micro-organisms indicates a similar behaviour towards cyanide.

Activated sludge of wastewater treatment plants not acclimated to cyanide is sensitive to the toxicity of cyanide. Cyanide concentrations in non-adapted facilities should not exceed 0.1 mg CN^-/l in order to avoid deterioration of the effluent quality. In purpose-designed systems, acclimated microbial consortia may be able to resist cyanide concentrations of 50 mg/l and more (Section 4.3.5, biodegradation).

Acute toxicity of cyanides to aquatic organisms, such as fish, invertebrates and algae, has been studied extensively. Acute toxicity to fish as 96-hour LC_{50} values ranged from 27 to 169 µg/l. The lowest value was obtained for rainbow trout (*O. mykiss*) under flow-through conditions. Saltwater species were generally less sensitive than freshwater species (≈ factor 2). Acute toxicity to invertebrates showed a wide variation between species and the values in the valid studies ranged from 30 µg/l to 2,000 µg/l. The lowest reliable EC_{50} value for *Daphnia* was 40 g/l. The lowest value obtained for the saltwater crab *Cancer irroratus* was 4 µg/l, but for other species of the genus *Cancer*, EC_{50} values above 30 µg/l were reported. The 24- or 96-h EC_{50} values for algae ranged from 45 to > 500 µg/l.

For chronic fish toxicity studies, reliable NOEC values ranged from 1 to 29 µg/l, for invertebrates from 3.9 to 30 µg/l approximately and for algae from 3.9 to 700 µg/l.

With regard to wildlife toxicity, birds (in particular Mallards), seem to be the most sensitive species. The toxicity of cyanides will, however, depend on the dose rate and the conditions of exposure. The steep dose-response curve and the relative quick recovery after sublethal doses of cyanide that were observed in 5-day studies with Mallards are in accordance with the results of studies in experimental animals, and confirm the predominance of acute toxic effects over possible effects of repeated exposure. Although a clear NOAEL could not be established in the existing studies, the LOEL of 21.2 mg CN^-/l based on reduced food and water intake of Mallards can be used as a starting point to derive a level that would probably not lead to significant loss of wildlife. For the field situation, ingestion of contaminated water is considered the main route of cyanide exposure in birds, although inhalation and dermal exposure cannot be excluded.

In the environment, plants (including their seeds and tubers) may be exposed to cyanide through air, soil and pore water. On the other hand, certain plants themselves may be sources of cyanide when cyanogenic glucosides are metabolised or under certain metabolic conditions.

In comparison to other organisms, plants seem to be relatively resistant to cyanides. For example, short-term exposure of roots to concentrations in excess of 1 mg/l in water will be tolerated in most cases. Dormant seeds and tubers show an even higher resistance if exposed by air or water. HCN has been successfully used at concentrations in excess of 1,000 ppm (1,124 mg/m^3) for the fumigation (elimination of parasitic insects) of different types of nutrient grain.

7. KINETICS AND METABOLISM

The toxicokinetics of cyanide are based on its physico-chemical properties.

HCN is a relatively weak acid, with a pKa value of 9.11 at 30°C (Table 1). This means that at the physiological pH of about 7, HCN is distributed in the body as such and is not present as CN^- ion. Hence, the form of cyanide, to which exposure takes place, i.e. a simple salt or the free acid, does not influence the distribution, metabolism or excretion from the body.

Blood has a limited capacity to bind cyanide by complex formation with methaemoglobin. The background concentration of methaemoglobin is about 0.3%, and blood methaemoglobin can complex about 2.4 mg CN^- in total or 0.6 mg CN^-/l blood. This might explain why any signs of intoxication are absent if the total concentration of cyanide in blood is below 600 µg/ml.

Commercial and technical grade ACH are stabilised by the addition of 0.01% sulphuric or phosphoric acid. Stabilised ACH will exert a significant vapour pressure, primarily due to the presence of more volatile HCN, at room temperature (Table 1). Under physiological conditions, acid-stabilised ACH will be buffered by the intracellular buffering capacity resulting in its rapid and quantitative decomposition to HCN and acetone (Section 4.3). Hence, ACH will exhibit the combined characteristics of HCN and acetone (Frank *et al*, 2002).

7.1 Absorption

Cyanides can be absorbed by inhalation, dermal uptake and ingestion.

7.1.1 Inhalation

In order to develop acute exposure guideline levels (AEGLs), the US-National Advisory Committee (NAC) has pointed to the water solubility of HCN and the ease of absorption in the moist respiratory tract (US-NAC, 2000a). On the basis of experimental observations, the retention of HCN in the nasal passages was reported to be between 13 and 23% (average 19%) and the retention in the lung from mouth breathing between 39 to 77% (average 61%). From this an average retention in the respiratory tract of 50% might be estimated (Landahl and Hermann, 1950). Probably the majority of the HCN retained in the respiratory tract is absorbed into the blood as it is likely to diffuse readily due to its water solubility, low molecular mass (size) and the fact that it is undissociated at cellular pH.

The Task Force has calculated the absorption on the basis of physico-chemical properties. The partition coefficient between blood and air is estimated to be 1 on the basis of vapour pressure and water solubility. Because the alveolar ventilation and the cardiac output (in l/h) are similar, it is estimated that 50% of the inhaled HCN is immediately absorbed in the blood. This agrees with the level of retention suggested by the AEGL-committee on the basis of observations of Landahl and Hermann (1950). The capacity of the lungs to metabolise HCN is assumed to be low, so a first-pass effect is low. HCN absorbed in the lungs is distributed to the tissues including the brain, which is extremely sensitive. At a level of 500 mg HCN/m^3 (445 ppm), assuming a ventilation rate of 0.02 m^3/min, 10 mg HCN/min is inhaled and 5 mg retained by the body. The methaemoglobin present may initially complex 2.4 mg but then the organism starts to be overloaded and free HCN increases in the plasma. The free HCN in plasma is distributed to the body tissues. In the body tissues cyanide binds intracellularly to cytochrome oxidase (Sections 7.3 and 8.0).

7.1.2 Dermal

The permeation of cyanide from aqueous solutions of NaCN through human skin *in vitro* was strongly influenced by the pH of the solution. HCN appeared to permeate 30 times faster than CN^- (Dugard, 1987). At a pH of 9.11 (= pKa) about 50% is present as undissociated HCN. The ratio of HCN and CN^- at a certain pH follows from the Henderson-Hasselbach equation (Eq. 32 and 33). Thus, the absorption rate through the skin can be calculated as follows (Eq. 34).

$$HCN = \frac{10^{-pH}}{10^{-pH} + 10^{-9.11}} \times CN_{total} \quad \text{..... (Eq. 32)}$$

$$CN^- = \frac{10^{-9.11}}{10^{-pH} + 10^{-9.11}} \times CN_{total} \quad \text{..... (Eq. 33)}$$

$$\text{Total CN permeation rate} = (3.5 \times 10^{-4} \times CN^- + 10^{-2} \times HCN) \times K_{skin\text{-}water\ [HCN]} \quad \text{..... (Eq. 34)}$$

Where total CN^- permeation rate is expressed in mg/cm²/h

HCN, CN^- and CN_{total} in mg/ml

Aqueous permeation coefficient: $K_{skin\text{-}water\ [HCN]}$ = 0.01 cm/h (Dugard, 1987)

To derive a dermal permeation coefficient for an airborne substance the following partitioning steps are taken into consideration: (i) from ambient air to a stagnant layer of air above the skin (default 3 cm simulating clothing), (ii) from stagnant air to water layer on the skin and (iii) from

the water layer into the skin. These three processes are described by the following equations (Wilschut and ten Berge, 1996).

Partition coefficient water-air:

$$K_{wa} = \frac{R \times T \times Sb}{Mw \times Vp} \quad \text{..... (Eq. 35)}$$

Where R = gas constant 8.314 Nm/mol/°K

T = temperature in °K

Sb = water solubility in mg/l

Mw = molecular weight (mass)

Vp = vapour pressure in Pascal (= N/m²)

Permeation coefficient skin-air: $Kp_{sk\text{-}air} = Kp_{sk\text{-}water} \times K_{wa}$ (cm/h) ..... (Eq. 36)

Permeation coefficient stagnant air layer: $Kp_{air} = D_{air}/\partial$ (cm²/h) ..... (Eq. 37)

Where ∂ = 3 cm (stagnant air layer, simulating barrier of clothing) (Lotens and Wammes, 1993)

$D_{air} = 360 \times \sqrt{76/Mw}$ (diffusivity in air, cm²/h)

The diffusivity of carbon disulphide (CS_2) in air has been given as 0.1 cm²/s (360 cm²/h) in former versions of the Handbook of Chemistry and Physics (Weast, 1971). However, one has to take into account a difference of molecular mass. The molecular mass of CS_2 is 76. So the correction for other substances is the square route of 76/Mw, where Mw is the molecular mass of the compound for which diffusivity has to be estimated.

The overall permeation coefficient ($Kp_{sk\text{-}air\text{-}air}$) for HCN is 6.25 cm/h, following

$$Kp_{sk\text{-}air\text{-}air} = \frac{1}{\frac{1}{Kp_{sk-air}} + \frac{1}{Kp_{air}}} \quad \text{..... (Eq. 38)}$$

Where $Kp_{sk\text{-}air\text{-}air}$ = 6.25 cm/h for HCN

From the dermal permeation coefficient in air, the total body surface and the duration of contact of the gas with the skin, the dermal absorption can be estimated and compared to the absorption via the lungs by inhalation.

$$\text{Ratio}\left(\frac{\text{skin absorption}}{\text{inhalation}}\right) = \frac{Kp_{sk-air-air} \times Ba}{Frt \times Va} \quad \text{..... (Eq. 39)}$$

Where Ba = surface area of the skin (18,000 cm²)
Va = alveolar ventilation (1,250,000 cm^3/h)
Frt = lung retention of inhaled HCN (0.5) (Landahl and Hermann, 1950) (Section 7.1.1)

It follows that the ratio absorption skin-lung for HCN is 6.25 × 18,000 / 0.5 × 1,250,000 = 0.18 or 18%.

Drinker (1932) reported that workers (3 men) equipped with respiratory protection (filter masks) felt symptoms of dizziness, weakness and strongly increased heartbeat, after exposure to 22,000 mg HCN/m^3 (19,600 ppm) for about 9 minutes. The Task Force has estimated from this simple observation the absorption of HCN through the skin, as follows. The LC_{01} at 10 minutes for humans has been estimated to be 211 mg/m^3 (188 ppm) (Section 8.1.5: Table 42). During normal activities workers inhale 1.25 m^3/h, or 0.21 m^3/10 min. Assuming a retention of HCN of 50% in the lungs (Landahl and Hermann 1950), the dose of HCN absorbed after 10 minutes exposure to 211 mg/m^3 is estimated to be about 22 mg. This can be assumed to be the amount of HCN that the workers reported by Drinker (1932) had absorbed through the skin when symptoms of HCN poisoning became evident after 9 minutes. Without respiratory protection, the workers (inhaling 0.19 m^3/9 min at 22,000 HCN mg/m^3 and assuming a lung retention of 50%) would have retained 2,090 mg HCN. Based on this calculation, the amount of HCN absorbed through the human skin (within 9 minutes) was as low as 1.3% of the respiratory absorption at a given concentration in air. This is more than one order of magnitude lower than the percentage of 18% estimated from Dugard (1987), and may be due to some lag time before HCN permeates the skin fully.

The example shows that respiratory protection alone does not protect workers exposed to high concentrations of HCN. This is borne out by industrial experience (Section 9.1.2).

Ballantyne (1994a) studied the absorption of HCN through rabbit skin, using a glass cell glued to the skin. The interpretation of this study is hampered by lack of information of the area of skin contact. This makes it impossible to estimate the dermal absorption rate per cm^2.

The capacity of the skin to metabolise HCN is assumed to be low, so a first-pass effect does not occur.

7.1.3 Oral

All cyanides ingested will be present, at the physiological pH of the stomach, as HCN**.** The HCN will be absorbed in the blood via the stomach wall and in the intestines. Before the blood enters the systemic circulation it has to pass the liver with a relatively high content of the enzyme rhodanese. A considerable part of cyanide is detoxified in the liver. This is apparent from comparison of the oral LD_{50} value for rabbits (0.092 mmol/kgbw) with those obtained by intravenous (*i.v.*) injection or inhalation for 5 minutes (0.022 or 0.019 mmol/kgbw, respectively) (Ballantyne, 1987a), indicating a first-pass effect via ingestion.

7.2 Distribution of hydrocyanic acid in the body

HCN is not equally distributed through the body. Bright and Marrs (1988) studied the toxicokinetics of KCN in Beagle bitches and found an apparent distribution volume of 0.209 l/kgbw.

A complete analysis of the body distribution in humans (3 fatally poisoned persons) showed that 50% of the absorbed cyanide was present in the blood, about 25% in the muscles and the remaining 25% in all the organs together. The apparent distribution volume was 0.075 l/kgbw (Schulz, 1984). Measuring total blood levels provides useful information on the total body burden of HCN.

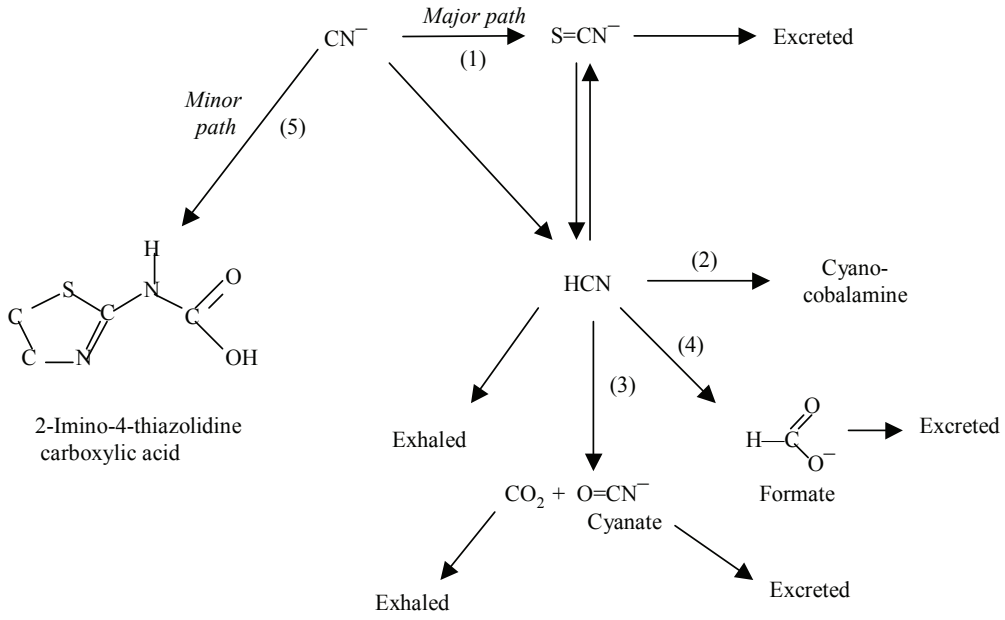
7.3 Biotransformation and excretion

Cyanides are metabolised via various pathways. The following pathways have been reported (the numbers are referred to in Figure 25).

1. Trans-sulphuration via the enzymes rhodanese or 3-mercaptopyruvate sulphotransferase, in which cyanide receives an atom of sulphur from thiosulphate and is converted into the less toxic thiocyanate (major pathway);
2. Reaction with hydroxocobalamin into cyanocobalamin (vitamin B_{12});
3. Oxidation to cyanate and carbon dioxide;
4. Hydrolysis into formate;
5. Reaction with cysteine into 2-imino-4-thiazolidine carboxylic acid.

Thiocyanate, cobalamin, formates and 2-imino-4-thiazolidine carboxylic acid are excreted via the urine. HCN and CO_2 are exhaled.

Figure 25: CN^- metabolism (adapted from Ansell and Lewis, 1970)



The main pathway of transformation of cyanide into thiocyanate is catalysed by the enzyme rhodanese, a mitochondrial enzyme that occurs in many tissues.

Rhodanese levels have been determined in several organs of a variety of species and were highest in liver, kidney, adrenals, thyroid and pancreas. Guinea pig and bovine livers had higher rhodanese contents than dog livers. Dog liver had sufficient rhodanese to convert about 243 mg CN^-/min to SCN^-; muscle (muscle weight ≈ 1 kg) could convert another 117 mg/min (Himwich and Saunders, 1948). Dahl (1989) observed that the rhodanese activity per gram tissue was 5 times higher in the nasal respiratory and olfactory mucosa than in the liver. Devlin *et al* (1989) found that the rate of cyanide clearance (ml/min/kg perfused tissue) was 20 fold higher in the liver than in the hind limbs. When the results of the hind limbs were extrapolated to the total body skeletal muscle mass, the rate of cyanide clearance by the total liver mass was only 1.5 times greater than in total muscle mass in the presence of thiosulphate.

The other enzyme capable of converting CN^- to thiocyanate has been identified as 3-mercaptopyruvate sulphotransferase in liver and erythrocytes of different species. Rats showed the highest levels. Levels in other species such as rabbits, dogs and pigs were 2 to 3 orders of magnitude lower. The 3-mercaptopyruvate sulphotransferase content of human erythrocytes was 20 times less than that in rat erythrocytes (Wood, 1975). Cyanide was catalysed into thiocyanate by 3-mercaptopyruvate sulphotransferase from bovine kidney extracts in the presence of

3-mercaptopyruvate, while rhodanese was inhibited by picrylsulphonic acid. The conversion rate into thiocyanate (per gram tissue extract) via both pathways was similar (Wing *et al*, 1992).

The distribution of the activity of rhodanese and 3-mercaptopyruvate sulphotransferase was studied in rat and human brain areas. Very low 3-mercaptopyruvate sulphotransferase activity was detected in rat and human brain. Rhodanese activity was widely distributed in all brain areas. In the rat, the olfactory bulb showed the highest rhodanese activity. Rhodanese activity was also high in the thalamus, septum, hippocampus and dorsal part of the midbrain, but low in the cerebral cortex. The distribution in human brain *post mortem* was essentially the same as in rat brain. Highest activity was found in thalamus, amygdala, centrum semiovale, colliculus superior, cerebellar cortex and spinal cord. Lowest activities were observed in the substantia nigra, pallidum and parts of the cerebral cortex (Mimori *et al*, 1984).

Blood samples from a randomly selected panel of 170 human subjects and *post mortem* tissues were investigated (by isoelectric focussing) for possible polymorphisms in rhodanese enzymes. The authors identified different isoenzymes in the different organs that fell into two main groups, a simple red cell type present in erythrocytes and all other tissues, and a more complex and more active tissue isoenzyme type present in many tissues other than erythrocytes. The highest enzyme activity was attributed to the isoenzymes in liver and kidney. No indications of an inherited polymorphism of rhodanese were observed (Whitehouse *et al*, 1988).

Serum rhodanese activity was determined in 14 healthy humans, 13 patients with pancreatitis and 12 patients with goitre. The age of the subjects was between 30 and 34 years. No significant difference of serum rhodanese activity was observed in patients with goitre (82 [range 50 - 144] µmol SCN/min/l) compared to controls (71 [range 22 - 160] µmol SCN/min/l). Significantly higher levels were observed in patients with pancreatitis (110 [range 64 - 180] µmol SCN/min/l). The latter finding was attributed to an induction of liver enzymes observed commonly in pancreatitis patients (Narendranathan *et al*, 1989).

Rhodanese activity in serum samples of 31 healthy humans was determined using methane thiosulphonate as a substrate. Normal values in the serum of males were reported to be 21.5 ± 6.4 U/l in males and 20.3 ± 12.7 U/l in females. The serum values were reported to be low compared to activities in erythrocytes and organs as liver and kidney. Elevated serum rhodanese levels were found in cases of liver diseases (Nawata *et al*, 1991).

Small American Indian and Eskimo populations with a genetic polymorphism of erythrocyte-rhodanese have been identified. Most of the individuals investigated had the same isoenzyme as non-Indian white people. No indications of the enzyme activities of the variants were given in the publication (Scott and Wright, 1980).

Schulz *et al* (1982) studied the release of cyanide as a side effect of medical treatment with sodium nitroprusside, $Na_2[Fe(CN)_5NO]$, as vasodilatory and hypotensive drug. Sodium nitroprusside decomposes fast and 44% of its weight is converted to cyanide. A number (51) of patients received *i.v.* infusion of sodium nitroprusside for up to 3 hours. The authors claimed that above an infusion rate of 100 to 150 µg sodium nitroprusside/min (≈ 2 µg/kgbw/min or 0.9 µg CN^-/kgbw/min) there was an increase of free cyanide levels in the blood that followed zero order kinetics caused by depletion of thiosulphate, i.e. the detoxification mechanism had been saturated due to a lack of the substrate thiosulphate. The authors also reported some biochemical disturbances (not specified) in the absence of clinical symptoms above 40 nmol CN^-/ml erythrocytes, and clinical symptoms above 200 nmol CN^-/ml erythrocytes (Schulz *et al*, 1982; Schulz, 1984). A quantitative evaluation of the data revealed a detoxifying rate of 0.003 mg/CN/kgbw/min for an 80-minute exposure period in humans (Appendix L).

The rate of biotransformation of cyanide via all detoxification routes was studied in Beagle dogs dosed with 0.3 to 0.4 mg KCN/kgbw (≈ 0.35 mg KCN/kgbw or 0.14 mg CN^-/kgbw) *i.v.* in about 20 seconds. In the period up to about 80 minutes after dosing, blood levels fell in a manner consistent with first order elimination kinetics. The half-life time of cyanide was about 24 minutes. The elimination rate constant appeared to be approximately 0.03/min or 1.8/h (Bright and Marrs, 1988). From these data a detoxification rate of 0.004 mg CN^-/kgbw/min in dogs was derived.

NaCN was applied via drinking water to F344 rats and $B6C3F_1$ mice (10/sex/species/group) for 13 weeks. At the highest dose level (300 ppm) rats absorbed an average dose of 12.5 mg CN^-/kgbw/d and mice an average dose of 26.5 mg/kg/d. Survival and bodyweight at this dose were similar to that of the control group (Hébert, 1993). These data suggested that the rat might detoxify at least 0.009 CN^-/kgbw/min and the mouse 0.018 CN^-/kgbw/min at non-toxic dose levels.

Leuschner *et al* (1991) studied the kinetics of cyanide in Sprague-Dawley rats (3 males/group) receiving by gavage a single dose of 1 mg KCN/kgbw (0.4 mg CN^-/kgbw) in an application volume of 10 ml/kgbw. Blood was collected by cardiac puncture at 2, 5, 10, 15, 30, 45 and 60 minutes following administration. A peak blood level of 6.2 nmol CN^-/ml (0.161 µg/ml) blood was observed 2 minutes after administration, after 60 minutes the blood cyanide level had dropped to below the detection limit of 0.3 nmol/ml. A blood elimination half-life of 14.1 minutes was calculated (Leuschner *et al*, 1991). From these data, a first-order rate constant of 0.05/min can be calculated. Assuming a blood volume of 64.1 ml/kgbw (Semler *et al*, 1992), this would correspond to a blood detoxification rate of 0.0005 mg CN^-/kgbw/min (excluding a first-pass effect). The overall detoxification rate would amount to 0.02 mg/kgbw/min (c_0 = 0.4 mg CN^-/kgbw/0.05).

The 13-week experiment was conducted in Sprague-Dawley rats (5 males/group) using dose levels of 0, 40, 80, 160 (reduced to 140 from week 12 due to observed sedation) mg KCN/kgbw/d in drinking water, corresponding to 0, 16, 32, 64 (56) mg CN^-/kgbw/d. Cyanide and thiocyanate levels were determined in plasma and urine of five animals in weeks 1, 3, 5, 7, 9, 11 and 13 (plasma) and in week 6 and 13 (urine). The urine was collected over a period of 16 hours. Cyanide blood levels remained relatively constant during the study, while the thiocyanate levels showed more variation, and in particular in the high-dose group decreased during the study. Thiocyanate excretion in urine increased with increasing dose. After 6 weeks, all dose groups excreted about 11% of the administered cyanide as thiocyanate in urine and approximately 0.003% as cyanide (Leuschner *et al*, 1991). Blood and urinary levels of cyanide and thiocyanate were provided by Winkler (2003) (Table 34).

The 13-week study of Leuschner *et al* (1991) corroborates the observation of other studies that, in the rat at dose levels upwards from about 10 mg CN^-/kgbw/d, there is less detoxification via thiocyanate than at lower dose levels. In the study of Hébert (1993), 60 to 100% of the dose was excreted as SCN^- at 4.2 mg CN^-/kgbw/d, while at 12.5 mg CN^-/kgbw/d urinary excretion of SCN^- only amounted to 7 to 22% of the dose. Urinary volumes were reduced considerably as well. It can be speculated that, when the thiocyanate pathway becomes saturated without a corresponding increase in CN^- blood concentration (Table 34), exhalation of HCN or other metabolic routes become more important in the rat. An overview of all available SCN^- levels in blood (serum) and urine is given in Appendix D.

Table 34: CN^- and SCN^- levels in Sprague-Dawley rats during 13 weeks of application in drinking water (Leuschner *et al*, 1991; Winkler, 2003)

Dose level (mg CN^-/kgbw)	Week	Blood		Urine	
		CN^- (µmol/ml)	SCN^- (µmol/ml)	CN^- (µmol/kgbw/24 h)	SCN^- (µmol/kgbw/24 h)
0 (control)	1	Not detectable	0.011		
	3		0.019		
	5		0.036		
	6		-	Not detectable	0.82
	7		0.054		
	9		0.049		
	11		0.036		
	13		0.039	Not detectable	0.77
16	1	0.0043	0.17		
	3	0.0037	0.23		
	5	0.0046	0.32		
	6	-	-	0.0196	68.6
	7	0.0026	0.26		
	9	0.006	0.23		
	11	0.0053	0.32		
	13	0.0073	0.19	0.0229	60.1
32	1	0.0079	0.28		
	3	0.0096	0.26		
	5	0.0098	0.42		
	6	-	-	0.0737	142.7
	7	0.0078	0.34		
	9	0.0141	0.37		
	11	0.0136	0.42		
	13	0.0136	0.40	0.0772	151.0
64/56	1	0.0055	0.24		
	3	0.0255	0.8874		
	5	0.0212	0.68		
	6	-	-	0.2416	197.5
	7	0.016	0.45		
	9	0.0192	0.34		
	11	0.0207	0.46		
	13	0.0238	0.48	0.1807	443.6

The lowest dose data indicate that at least 16 mg CN^-/kgbw/d are detoxified, resulting in a detoxification rate of approximately 0.01 mg CN^-/kgbw/min. This is comparable to the detoxification rate of 0.009 mg CN^-/kgbw/min in male rats derived from the above study of Hébert (1993).

Another possibility for investigating the detoxifying rate is to look at the acute inhalation studies in rats, reported by Ballantyne (1994b). The LC_{50} 5 minutes was estimated to be 449 mg/m^3 and the LC_{50} 60 minutes 144 mg/m^3. The detoxifying rate of cyanide is estimated from the following assumptions: breathing rate of rats 48 l/kgbw/h or 0.8 l/kgbw/min; absorption rate of HCN in the lungs 50%. The absorbed dose is estimated to be 0.9 mg/kgbw in 5 minutes and 3.4 mg/kgbw in 60 minutes. The difference of 2.6 mg/kgbw is detoxified over 55 minutes, i.e. 0.045 mg/kgbw/min. From the data of free moving rats of Barcroft (1931) a detoxification rate in rats of 0.029 mg/kgbw/min was calculated in the same manner (Table 41).

The same calculative approach can be made on the basis of the LC_{50} values for humans, estimated from the data of Barcroft (1931) for goats and monkeys (Section 8.1.5: Table 42). The following assumptions are made: LC_{50} human is 676 mg/m^3 for 5 minutes, LC_{50} human is 202 mg/m^3 for 60 minutes, human breathing rate (at rest) is 7.5 l/min (US-EPA, 1988), absorption of inhaled cyanide is 50% and human body weight is 70 kg. In the case of the LC_{50} of 5 minutes, a human being absorbs about 12.7 mg CN^- and in case of the LC_{50} of 60 minutes about 45 mg. The difference of 32.3 mg might be detoxified in 55 minutes. In one hour, 35.2 mg cyanide is detoxified (0.59 mg/min) under acute lethal concentrations, i.e. for a 70 kg human 8.4 µg CN^-/kgbw/min is detoxified. Considering the steep acute dose-response for cyanide toxicity and the imprecision of the estimate, this is broadly comparable to the detoxifying capacity of cyanide in patients infused with sodium nitroprusside derived from the data of Schulz *et al*, 1982; Schulz, 1984 at the level approaching lethality (3.7 µg CN^-/kgbw/min) (Appendix L).

7.4 Summary and evaluation

The predominant speciation of all inorganic cyanide in the body is HCN due to the weak acid dissociation constant (pKa = 9.11). This is also the form in which cyanide is absorbed into the blood after oral, dermal or inhalation exposure. It is rapidly absorbed via all exposure routes. The main route of metabolism is enzymatic (rhodanese) trans-sulphuration into thiocyanate. The liver metabolises nearly all cyanide at subtoxic dose levels via the enzyme rhodanese. So there is a first-pass effect via the oral route. However, the overall maximum detoxification capacity in humans derived from the difference in acute toxicity data at different exposure times is limited to about 0.008 mg CN^-/kgbw/min. Detoxification rates for other species ranged between 0.01 and 0.03 mg CN^-/kgbw/min and were thus a little higher.

The detoxifying enzyme rhodanese is not only found in the liver but also in muscles and other tissues. Rhodanese in muscles contributes considerably to the detoxification of cyanide in the body to thiocyanate. In the absence of sulphur donating agents in the human body, the maximum detoxification rate was claimed to be as low as 0.9 µg CN^-/kgbw/min (Schulz *et al*, 1982).

However, a re-analysis of the data suggests a rate of 3.0 µg CN^-/kgbw/min (80 min mean infusion duration), based on the dose rate at which no clinical symptoms occurred.

Inter-individual variation in serum rhodanese activity can vary by a factor of 6 (Nawata *et al*, 1991) or 3 to 8 (Narendranathan *et al*, 1989). However, rhodanese is present in all body tissues in considerable excess and not rate-limiting (Himwich and Saunders, 1948; Schulz *et al*, 1982), unlike thiosulphate, which may be only available in the body in small amounts depending on the nutritional status (Schulz *et al*, 1982). No major polymorphisms have been identified to date. A rare hereditary disease, Leber's optic atrophy has been linked by some authors to a deficiency in rhodanese activity (Cagianut *et al*, 1984; Wilson, 1965, 1983; Poole and Kind, 1986), but this was not confirmed by other authors (Pallini *et al*, 1987; Berninger *et al*, 1989; Whitehouse *et al*, 1989). Protein deficient populations are more susceptible to cyanide intoxication as thioamino acid levels are reduced (see also Chapter 9).

Dermal absorption of HCN vapour can be significant and has been estimated to be 18% of the inhaled amount, although calculations presented here suggest a ratio of one order of magnitude lower.

8. EFFECTS ON EXPERIMENTAL ANIMALS AND *IN VITRO* TEST SYSTEMS

8.0 Introduction

8.0.1 Mode of action

Cyanide poisoning is caused by complex formation with the iron in cytochrome oxidase which is present in tissues at cellular level. The complex formation inhibits oxygen from receiving electrons from the cytochrome oxidase and a so-called intracellular or cytotoxic anoxia occurs, i.e. oxygen is present but cannot be utilised by the cell (Section 8.0.2).

Because neurons and cardiac myocytes are highly dependent on aerobic metabolism they are extremely sensitive to the deprivation of oxygen. If aerobic metabolism fails due to the inactivated cytochrome oxidase by cyanide, the neuron immediately loses its capacity to conduct nervous pulses properly and the brain fails to function with consequent loss of consciousness. If this stage continues for some minutes, the damage becomes irreversible and the neurons die. For these reasons, prolonged hypoxia, regardless of its cause, often results in injury to the brain. Toxicants that inhibit aerobic cell respiration like HCN and hydrogen sulphide have the same effect (Anthony and Graham, 1991).

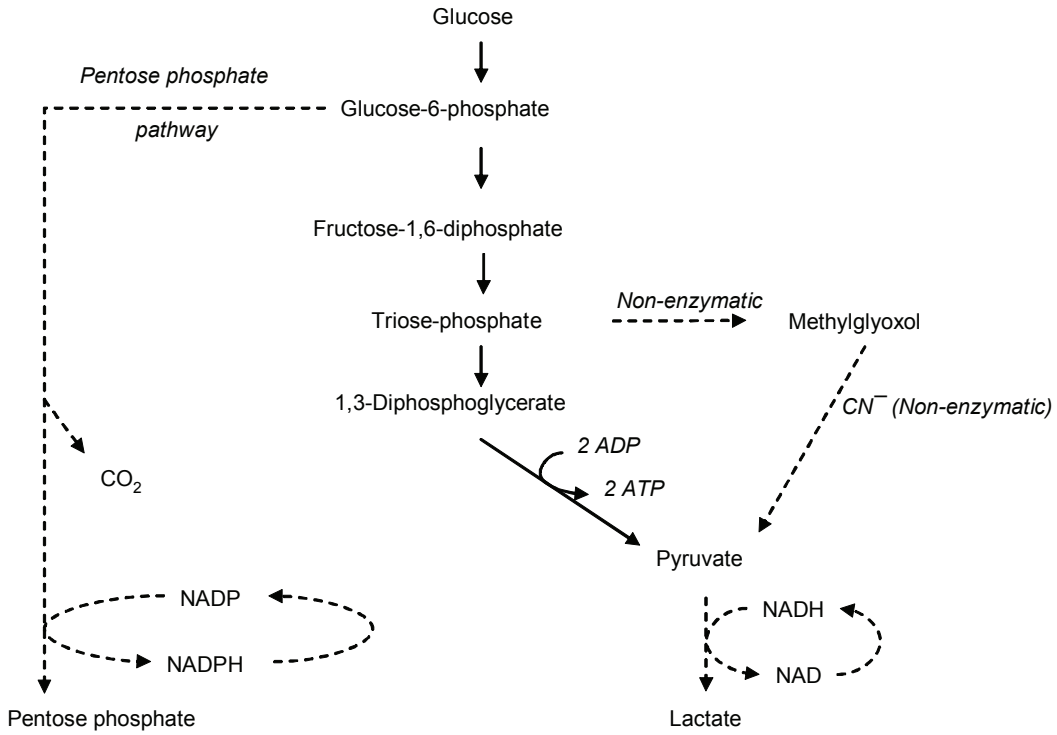
8.0.2 Molecular mechanism of action

Hypoxic anoxia

Over the last 6 decades, a number of authors have worked on the identification of the molecular mode of action of cyanide poisoning. In this evaluation emphasis is placed on the more recent work, in particular of the group of Isom (Isom *et al*, 1975, 1982) on the molecular basis of sub-lethal cyanide intoxication that has been conducted using model cell cultures of neuronal cells (e.g. undifferentiated or differentiated rat phaeochromocytome PC12 cells, cerebellar granular cells), rodent brain slices and *in vivo* experiments. These have elucidated a complicated mode of action that is illustrated in brief below. The key papers are reviewed in more detail in Appendix E.

The role of cytochrome oxidase inhibition and depletion of ATP energy equivalents has been identified relatively early (Albaum *et al*, 1946; Johnson *et al*, 1987a). Brain and liver are the main target organs for cytochrome *c* inhibition and ATP depletion (Isom *et al*, 1982). This leads to an inhibition of oxidative glucose metabolism in favour of anaerobic pathways, such as the pentose-phosphate shunt and non-enzymatic pyruvate formation, and results in an increased formation of NADPH and lactate (Figure 26) (Isom *et al*, 1975).

Figure 26: Proposed scheme[a] of carbohydrate metabolism in the presence of CN^-
(Isom *et al*, 1975)



[a] Broken lines represent metabolic pathways activated and/or increased in utilisation by CN^-. NAD(P)H = reduced nicotinamide adenine dinucleotide (phosphate)

At the cellular level, ATP depletion leads to an inhibition of Ca^{2+} and Ca^{2+}/Mg^{2+} dependent K-Na-ATPases that regulate, *inter alia*, cytosolic levels of calcium ions. A dose-dependent increase in cytosolic calcium levels occurs with a certain time delay indicating a metabolic process rather than a direct interaction with ion channels. Most of the effects that follow can, at least partially, be suppressed by calcium antagonists.

More recent studies have shown that cyanide may increase intracellular calcium levels by two additional mechanisms. Initially, intracellular calcium may be mobilised by a reaction activated by phospholipase C, leading to formation of polyphosphoinosite hydrolysis and inositol triphosphate (IP_3). Low levels of cyanide very rapidly resulted in elevated IP_3 levels in a cell culture system. IP_3 is known to mobilise Ca^{2+} from intracellular storage compartments (Yang *et al*, 1996). A delayed calcium influx might be mediated by the N-methyl-D-aspartate (NMDA) receptor channel. Initial mobilisation of intracellular calcium in brain cells leads to an increased release of glutamic acid, an excitatory central neurotransmitter. Increased calcium influx through glutamate gated NMDA-receptor channels leads to a prolonged release of glutamic

acid. High concentrations of glutamic acid can, in turn, induce neuronal degeneration due to excessive calcium influx. NMDA-mediated calcium influx is also triggered via IP_3 and a receptor-mediated phospholipase C (Patel *et al*, 1991; Yang *et al*, 1996).

Increase of cytosolic calcium triggers a number of other processes that have been observed following cyanide exposure.

1. Formation of reactive oxygen species (ROS) and NO is mediated by an activation of phosphokinase C (PKC). PKC activates phospholipase A2 (PLA2) and NO synthetase. The latter leads to an increased NO formation. PLA2 activates the arachidonic acid cascade and both cyclo-oxygenase (in particular cyclo-oxygenase-2) and lipoxygenase are activated, leading to increased levels of ROS (Gunasekar *et al*, 1998) in the endoplasmic reticulum (Kanthasamy *et al*, 1997). The elevated ROS and NO levels can explain the increased lipid peroxidation that was observed by several authors following cyanide treatment (Johnson *et al*, 1987a; Ardelt *et al*, 1989, 1994) in particular in the endoplasmic reticulum membranes (Ardelt *et al*, 1994). The elevated ROS and NO levels also underlie formation of malone dialdehyde resulting in cytotoxicity and apoptotic cell death (Mills *et al*, 1996).

2. Differentiated neuronal cells have been demonstrated to be more sensitive to ROS-induced apoptotic cell death following cyanide exposure than undifferentiated neuronal cells (Mills *et al*, 1996).

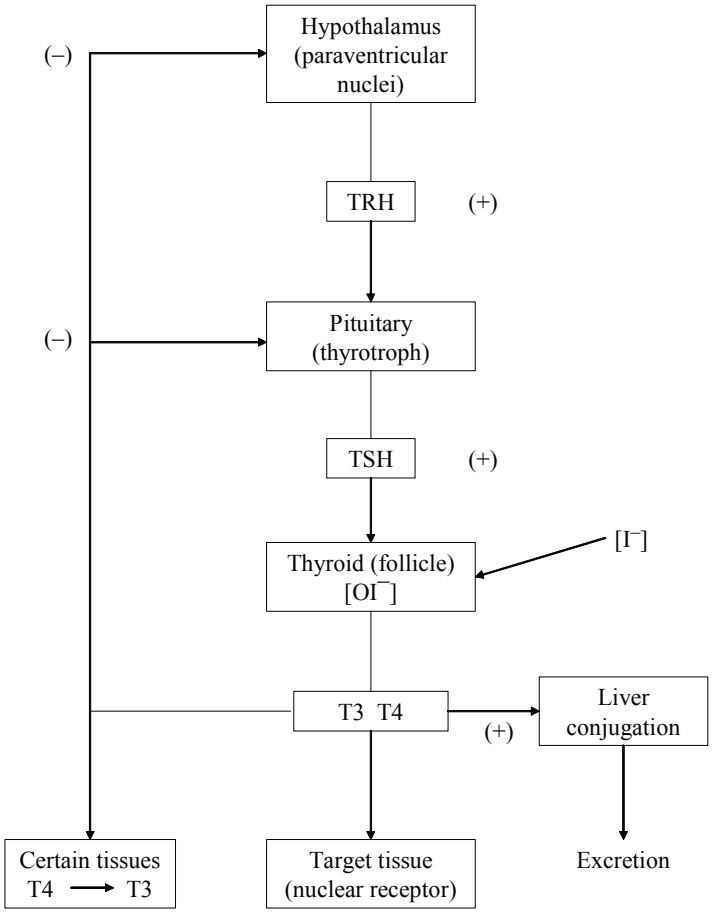
3. Cyanide-mediated elevated intracellular calcium levels lead to an activation of brain xanthine oxidase, thereby inducing an inhibition of antioxidative enzymes such as catalase, GSH-dependent peroxidase and GSH reductase, and consequently depletion of reduced glutathione (GSH) (Ardelt *et al*, 1989). This implies that the cells' ability to neutralise increased levels of ROS is also impaired adding to the effect of lipid peroxidation as described under 1.

4. Elevated intracellular calcium levels also cause the increased release of excitatory neurotransmitters like dopamine and noradrenaline from granular stores within neuronal cells in brain and periphery (Kanthasamy *et al*, 1991a). Adrenal catecholamine secretion may also be enhanced as a secondary effect (Borowitz *et al*, 1988). This contributes to an increased energy demand of the organism that aggravates the consequences of cyanide-induced ATP reduction and leads to a spread of the cytotoxic effect to other energy demanding organs, such as the heart which are less sensitive to the primary effect of cyanides.

Thiocyanate mediated

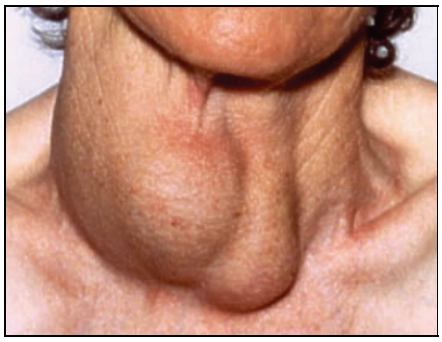
The secondary mechanism of action relevant to repeated exposure to cyanide is formation of the primary metabolite thiocyanate. The main target for thiocyanate is the thyroid gland. Thiocyanate is a competitive inhibitor of the iodine uptake by the thyroid, causing depletion of iodine in the thyroid depending on the ratio of thiocyanate and iodine levels in blood. Reduced intra-thyroidal iodine levels result in reduced thyroid hormone synthesis, excretion and blood levels, in particular tri-iodothyronin (T3) and serum thyroxin (T4). The lower T3 and T4 levels, in turn, up-regulate the hypothalamus, increasing secretion of thyrotropin releasing hormone (TRH). This leads to an increased secretion of thyroid stimulating hormone (TSH) by the pituitary and an increase in hormone producing cells of the thyroid that may result in hyperthyroidism (goitre) formation (Section 9.2 and 10), as illustrated in Figure 27 and 28.

Figure 27: Hypothalamic pituitary thyroid axis[a] (Hill, 1998)



[a] Abbreviations in text

Figure 28: Goitre (Parker, 2005)



8.1 Acute toxicity

In this section, an overview is given of the acute toxicity data of HCN, NaCN and KCN with emphasis on the effects caused by inhalation (HCN is a very toxic compound by this route (Section 8.1.3). The acute toxicity of ACH is also presented.

On a molar basis, there is no significant difference in acute toxicity between HCN, or its alkali salts, and ACH. This is consistent with the molar decomposition of ACH into acetone and HCN (US-NAC, 2000a,b).

8.1.1 Oral

Ballantyne (1987a) compiled oral acute toxicity data on HCN, NaCN and KCN for rats, rabbits and mice (Table 35). In general, the molar lethal oral LD_{50} values for these species appear to be quite similar. The rabbit seems to be more sensitive than rats. The degree of starvation has no consistent influence on the toxicity.

The acute toxicity data for ACH (Table 35) were derived from the updated 'international uniform chemical information database' (IUCLID) data set (Degussa, 1998). On a molar basis, the toxicity of HCN, NaCN, KCN and that of ACH are considered as comparable (within experimental error).

Table 35: Oral toxicity

Species (state) / Compound	LD_{50} (mg/kgbw)	LD_{50} (mmol/kgbw)	Reference
Rat (starved)			
HCN	3.62	0.127	Ballantyne, 1984 [a]
NaCN	5.09	0.104	Ballantyne, 1984 [a]
KCN	9.69	0.149	Ballantyne, 1984 [a]
Rat (unstarved)			
HCN	4.21	0.156	Ballantyne, 1984 [a]
NaCN	5.72	0.117	Ballantyne, 1984 [a]
KCN	7.49	0.115	Ballantyne, 1984 [a]
ACH	16	0.188	Smyth *et al*, 1962 [b]
Mouse			
ACH	14	0.164	Marhold, 1972 [b]
Rabbit (unstarved)			
HCN	2.49	0.092	Ballantyne, 1984 [a]
NaCN	5.11	0.104	Ballantyne, 1984 [a]
KCN	5.82	0.090	Ballantyne, 1984 [a]
ACH	13.5	0.159	Shkodich, 1966 [b]

[a] Cited by Ballantyne, 1987a; CoR 4b
[b] Cited by Degussa, 1998; CoR 4b

8.1.2 Dermal

Ballantyne (1987b) evaluated the dermal toxicity of HCN, NaCN and KCN under variable conditions in rabbits (Table 36). In addition, two studies are available with 10% ACH dissolved in water, both conducted according to OECD guideline 402 (OECD, 1987).

Table 36: Dermal toxicity in rabbits

Compound (form) / Skin condition	LD_{50} (mg/kgbw)	LD_{50} (mmol/kgbw)	Reference [a]
HCN (solution)			
Intact	6.89	0.260	Ballantyne, 1984 [a]
Abraded	2.34	0.087	Ballantyne, 1984 [a]
KCN (solution)			
Intact	22.3	0.343	Ballantyne, 1984 [a]
Abraded	14.3	0.220	Ballantyne, 1984 [a]
NaCN (solution)			
Intact	14.6	0.30	Ballantyne, 1984, 1986 [a]
Abraded	11.3	0.23	Ballantyne, 1984, 1986 [a]
NaCN (moist)			
Intact	11.8	0.24	Ballantyne, 1984, 1986 [a]
NaCN (powder)			
Intact	> 200	> 4.08	Ballantyne, 1984, 1986 [a]
Abraded	7.7	0.16	Ballantyne, 1984, 1986 [a]
ACH (solution)			
Intact	16.0	0.188	Carreon *et al*, 1981 [b]
Intact	850	10	Sterner and Chibanguza, 1980 [b]
Abraded	150	1.76	Sterner and Chibanguza, 1980 [b]

[a] Cited by Ballantyne, 1987a; CoR 4b
[b] CoR 2b

The dermal toxicity of HCN seems to be slightly greater than that of NaCN and KCN, especially in the case of abraded skin. Abrading the skin enhances the penetration of cyanide and increases lethal toxicity. Times to the onset of signs of toxicity following the application of solutions to the skin varied between 5 minutes and 1 hour with HCN. In the case of abraded skin, the times to intoxication were considerably shorter. With NaCN and KCN, the 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. Ballantyne (1987b) developed special equipment for exposure of the rabbit skin. This became clear from a more recent paper of the same author reporting the same results (Ballantyne, 1994a). Unfortunately, the author did not report the exposed surface area of the skin, but it was presumably no more than a few percent of the total body surface of the rabbit (total 1,270 cm^2, exposed 12 cm^2, estimated from a photograph in the paper). These dermal LD_{50} values were not generated following the OECD 402 protocol.

The difference between the two dermal LD_{50} values reported for ACH is remarkable since both studies were carried out according to the OECD 402 protocol, the only apparent difference being that Sterner and Chibanguza (1980) clipped the hair of only 150 cm^2 on the back, whereas Carreon *et al* (1981) clipped the hair from the full trunk of the rabbits. This suggests that the area of skin contact and integrity of the skin areas very important in case of ACH. Abrasion of the skin enhances the dose rate and increases the toxicity of ACH.

8.1.3 Inhalation toxicity

The AEGL-Committee (US-NAC, 2000a) evaluated the acute toxicity of HCN by inhalation in three species. The summary table from this review is represented in Table 37.

Table 37: Acute inhalation toxicity (US-NAC, 2000a)

Concentration		Exposure time	Parameter	Observation period (d)	Reference [a]
(mg/m^3)	(ppm) [b]				
Rat					
3,438	3,864	10 s	LC_{50}	-	Ballantyne, 1983
1,339	1,505	1 min	LC_{50}	-	Ballantyne, 1983
503	565	5 min	LC_{50}	7	Higgins *et al*, 1972
484	544	5 min	LC_{50}	-	Vernot *et al*, 1977
449	505	5 min	LC_{50}	-	Ballantyne, 1983
369	415	15 min	LC_{50}	14	Du Pont, 1981
196	220	30 min	LC_{50}	14	Du Pont, 1981
200	225	30 min	LC_{50}	-	Kimmerle, 1974
173	194	30 min	LC_{50}	14	Du Pont, 1981
157	176	30 min	LC_{50}	-	Ballantyne, 1983
110 [c]	124	30 min	LC_{50}	-	Levin *et al*, 1987
144	162	1 h	LC_{50}	-	Ballantyne, 1983
139	156	1 h	LC_{50}	14	Du Pont, 1981
120	135	1 h	LC_{50}	-	Kimmerle, 1974
68	76	6 h	LC_{30}	-	Blank, 1983
Mouse					
323	363	5 min	LC_{50}	7	Higgins *et al*, 1972; Vernot *et al*, 1977
166	187	30 min	LC_{50}	-	Matijak-Schaper and Alarie, 1982
150	169	4 h	LC_{100}	10	Pryor *et al*, 1975
100	112	4 h	LC_{10}	10	Pryor *et al*, 1975
100	112	12 h	LC_{100}	10	Pryor *et al*, 1975
Rabbit					
2,213	2,487	45 s	LC_{50}	-	Ballantyne, 1983
372	418	5 min	LC_{50}	-	Ballantyne, 1983
189	212	35 min	LC_{50}	-	Ballantyne, 1983

[a] Cited by US-NAC, 2000a; CoR 4b
[b] Converted (Section 2.4)
[c] Head-only

For ACH, the AEGL Committee found that hardly any data exist on inhalation. They relied on a range-finding study of Smyth *et al* (1962; CoR 2a) (Table 38).

Table 38: ACH Acute inhalation toxicity in rats (Smyth *et al*, 1962)

Concentration		Exposure time (h)	Number of deaths/exposed	Observation period (d)
(ppm)	(mg/m^3) [a]			
62.5	(221)	4	2/6	14
125	(442)	4	6/6	14

[a] Converted (Section 2.4)

8.1.4 Discussion: concentration-time relationship

The Task Force has explored in more depth the relation between inhaled dose (concentration over time, $C \times t$) and mortality, to gain a better understanding of acute inhalation hazard in humans. Special attention was given to the animal studies of Barcroft (1931), Lapin and Mackay (1981) and Ballantyne (1994b). The basic design of these studies was quite similar in that groups of 2 to 10 animals were exposed to a range of concentrations and exposure periods. During an exposure period, the atmospheric concentration was kept broadly constant. The Task Force then addressed the relative sensitivity of animals and humans with a view to deriving $C \times t$ dependent estimates of LC_{50} and LC_{01} values for humans. Barcroft (1931), though dated, is the only paper providing directly comparable information on species sensitivity.

Barcroft (1931) exposed goats, monkeys, rabbits, rats, cats, mice and dogs to HCN vapour in a large closed chamber and studied survival at different concentrations and exposure periods. Exposure levels were not measured, but otherwise the paper contains sufficient details of study design and equipment. The exposure chamber was described as a closed gas chamber with a volume of 10 m^3 and equipped with an electric fan stirring the air. An operator, provided with a respirator, went inside (through an air lock of about 2 m^3) with a calibrated amount of HCN in a glass tube and started the experiment by breaking the glass tube. The HCN evaporated immediately and the operator enhanced mixing of the air by waving a large piece of cardboard. The HCN concentration appeared to be decreased by 22% after 38 minutes. The period of observation of the animals after exposure was not mentioned. The exposure data of Barcroft (1931) were tabulated in a review by McNamara (1976).

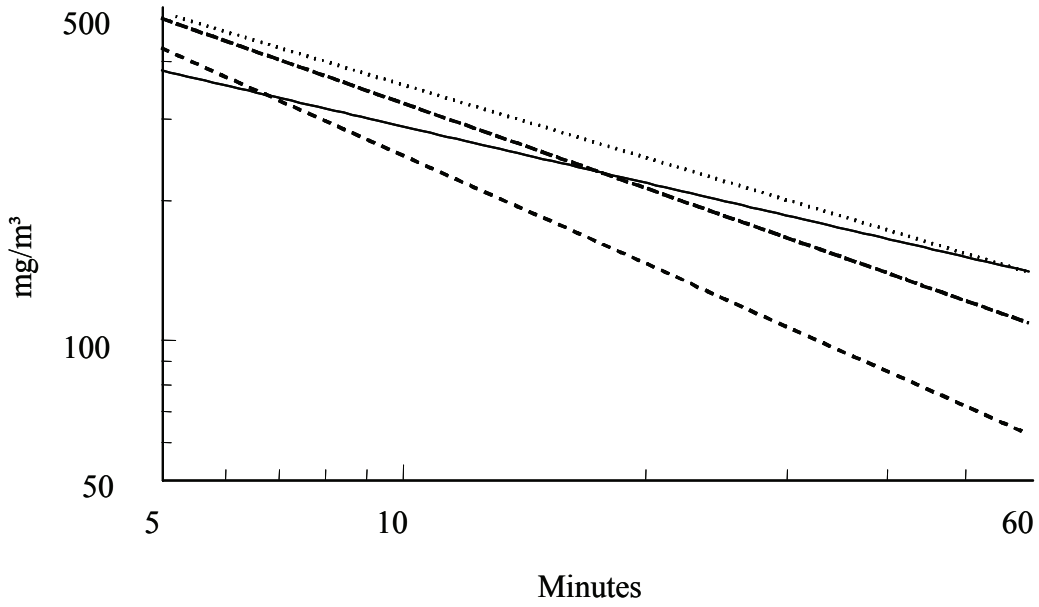
Lapin and Mackay (1981) exposed rats in a chamber (volume 175 l) that was continuously flowed through with HCN from a gas bottle, diluted with air. The front panel of the chamber was provided with exposure ports, in which animal holding tubes for nose-only exposure were fitted. The chamber atmosphere was measured continuously by infra-red spectrometry and validated by GC. Rats were exposed free-moving or under restraint (nose-only). Rats generally died during exposure. Survivors were followed for 7 days and body-weight gain was recorded.

Ballantyne (1994b) generated different HCN vapour concentrations by varying the ratio of mixing solutions of NaCN and HCl and evaporating the HCN by means of an air stream. Exposure concentrations were monitored by aspirating chamber air through bubblers (5 ml NaOH solution) at a flow rate of 1 l/min and determining cyanide in the alkaline washing liquid using a calibrated cyanide-specific electrode. The period of observation of the animals after exposure was not indicated. For deriving the concentration-time relationship for 50% mortality response, only those trials with an exposure period of more than one minute were used.

Analysis of the available data

In order to check the quality of the data of Barcroft (1931) the concentration-time mortality response relationship for rats was derived according to Finney (1977). The relationship of Barcroft was compared with the relationship derived from the extensive data sets of Lapin and Mackay (1981) and of Ballantyne (1994b) for rats. Figure 29 shows that there was no significant difference between the dose-mortality response of the free moving rats in all three studies. The restrained rats (Lapin and Mackay, 1981) appeared to be more sensitive than free moving rats.

Figure 29: Concentration-time relationship in rats for 50% mortality by HCN



[a] ------ Barcroft, 1931 (free moving), —— Lapin and Mackay, 1981 (free moving), - - - - Lapin and Mackay, 1981 (nose-only), ... Ballantyne, 1994b (free moving)

The LC_{50} (60 minutes) value for rats with nose-only exposure appeared to be 62.7 mg/m^3 (95% confidence limit 48.8 - 77.0) and for free moving rats 140 mg/m^3 (95% confidence limit 130 - 150). This difference is statistically significant and greater than a factor 2 (Lapin and Mackay, 1981).

The regression coefficients that characterise the probit equations of the concentration-time mortality response relationships in rats are presented in Table 39.

Table 39: Probit [a] equations of the concentration-time mortality response relationships in rats

Study	b	N	a
Barcroft, 1931 (free moving)	0.70	1.64	3.27
Lapin and Mackay, 1981 (free moving)	2.27	2.48	32.1
Lapin and Mackay, 1981 (nose-only)	2.46	1.29	18.3
Ballantyne, 1994b (free moving)	1.20	1.92	11.3

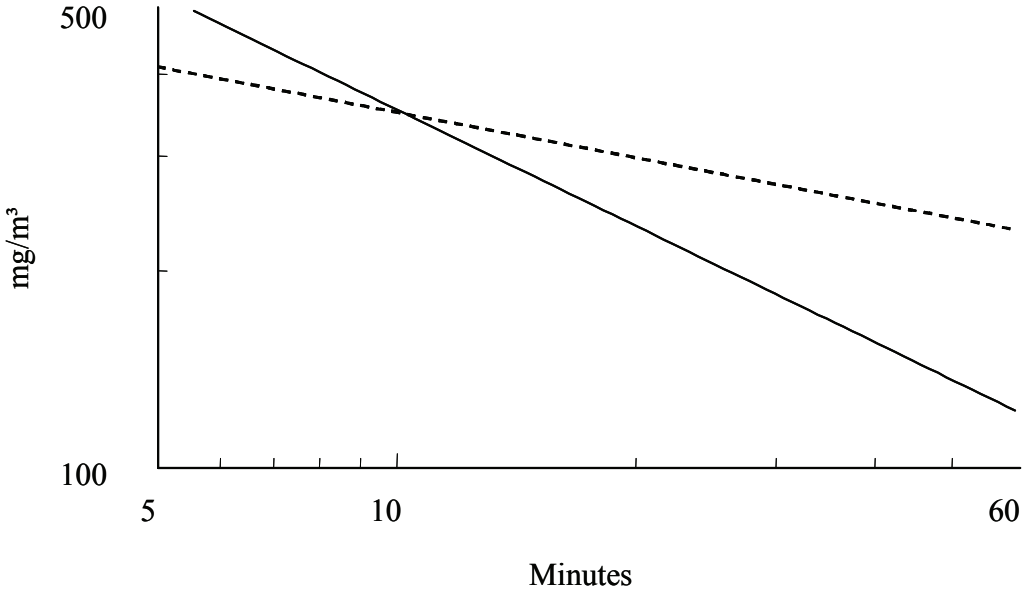
[a] Probit = $b \times \ln (C^N \times t) - a$; where b = slope, ln = natural logarithm, C = concentration, N = exponent, t = time, a = intercept

The table shows that the correlation derived from the observations of Barcroft (1931) is consistent with more recent studies, in which the concentrations were monitored during exposure.

Barcroft (1931) and Ballantyne (1994b) also exposed rabbits and a concentration-time mortality response relationship was derived for both data sets. The relationship is presented in Figure 30 and Table 40 below.

Figure 30: Concentration-time relationship in rabbits for 50% mortality by HCN

[a] - - - - Barcroft, 1931 (free moving), —— Ballantyne, 1994b (nose-only)



The LC_{50} (60 minutes) for restrained (nose-only exposed) rabbits (Ballantyne, 1994b) appeared to be 122 mg/m^3 (95% confidence limit 70 - 200) and for free-moving rabbits 231 mg/m^3 (95% confidence limit 177 - 354). The difference is not statistically significant, but is about a factor 2. This is similar to that found for free moving and nose-only exposed rats (Lapin and Mackay, 1981). The early data of Barcroft (1931) fit surprisingly well with the more recent data on acute toxicity of HCN in mammals, as shown by the coefficients of the regression equations [probit = $b \times \ln (C^N \times t) - a$] for rabbits (Table 40).

Table 40: Probit equations of the concentration-time mortality response relationships for rabbits

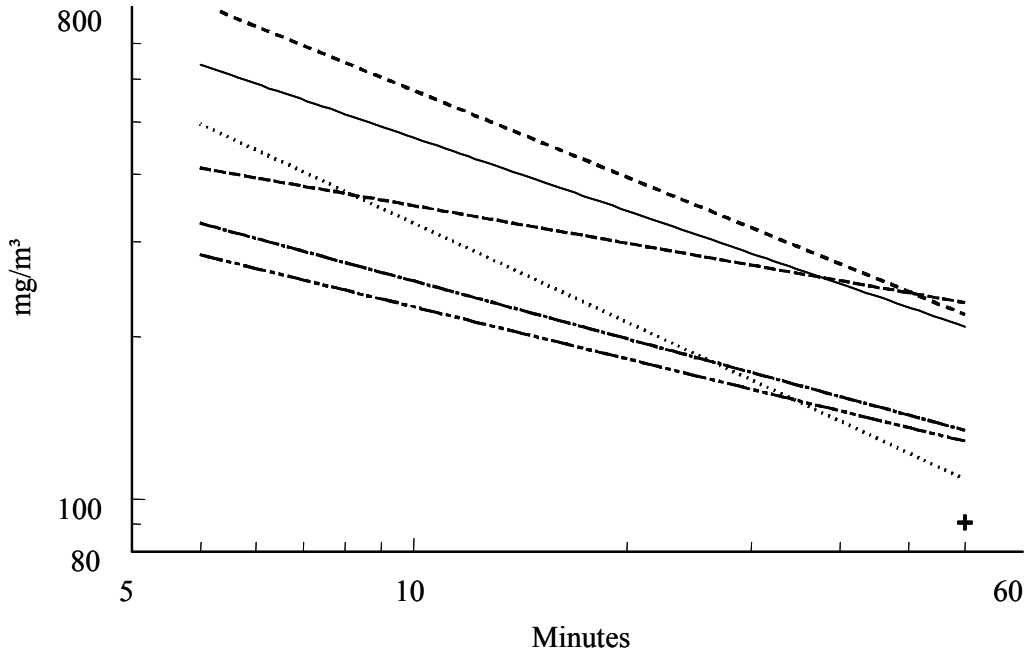
Study	Regression coefficient		
	b	**N**	**a**
Barcroft, 1931 (free moving)	0.744	4.33	15.6
Ballantyne, 1994b (nose-only)	1.20	1.92	11.3

As the concentration-time mortality response relationships for rats and rabbits observed by Barcroft (1931) are comparable to those of recent studies that included monitoring of the atmospheric HCN, it is reasonable to assume that the data for the other species included in the Barcroft (1931) publication are also reliable.

Differences in species sensitivity to acute inhaled HCN

Derived concentration-time mortality response relationships for the 7 species studied by Barcroft (1931) are presented in Figure 31 and Table 41 below.

Figure 31: Concentration-time relationship, 50% mortality[a] (Barcroft, 1931)



[a] - - - - Monkey, —— goat, ... rat, ----- rabbit, – · – · – cat, – ·· – ·· – dog, + mice

Goats and monkeys appeared to be least sensitive and mice, cats and dogs most sensitive to inhaled HCN. It was not possible to derive a concentration-time mortality response relationship for mice, because the data were too limited for short exposure durations. Only the LC_{50} value at 60 minutes was plotted (+ in Figure 31).

The coefficients of the regression equations [probit = $b \times \ln (C^N \times t) - a$] are presented in Table 41 below.

The exponent N describing the effective lethal dose ($C^N \times t$) in monkeys (N = 1.88 for the observations of Barcroft, 1931) is similar to the exponent N for the description of the effective dose for incapacitation (N = 2.08), as can be derived from the time to incapacitation of monkeys at variable exposure levels of HCN (Purser *et al*, 1984).

8.1.5 Evaluation of the acute inhalation data in predicting hazard to humans

The derived data in Figure 31 appear to show a trend of larger species showing lower sensitivity to inhaled cyanide than smaller species. However, dogs and cats do not fit this trend well. The Task Force estimated the detoxifying capacity per kg body weight per minute from the LC_{50} at 65 minutes and the LC_{50} at 5 minutes exposure (Appendix F). The difference in absorbed amount of HCN between 65 and 5 minutes was assumed to be detoxified and was compared with the respiratory volume per kg body weight and hour for each species [data taken from Snipes (1989) except for goats where respiratory volume was calculated from the average tidal volume of 7.43 l/min reported by Santiago and Edelman (1976) and an assumed body weight of 30 kg for an adult goat]. This is shown in Table 41. The comparison shows that the derived detoxification rates are quite similar in different species with rates between 0.015 (cat) and 0.029 mg CN^-/kgbw/min (rat).

Table 41: Regression coefficients for free moving animals (Barcroft, 1931) and estimates of respiratory volume and detoxifying capacity

Species	Regression coefficient			LC_{50} (mg/m^3)		Respiratory volume (l/kgbw/h)	Detoxifying rate (mg CN^-/kgbw/min)
	b	N	a	5 min	65 min		
Goat	2.024	2.22	27.3	638	201	15	0.021
Monkey	0.835	1.88	6.87	829	211	17	0.023
Rabbit	0.744	4.33	15.6	411	227	16	0.028
Rat	0.701	1.64	3.27	496	104	48	0.029
Cat	0.741	2.81	8.26	325	131	16	0.015
Dog	0.327	3.13	1.3	283	125	22	0.021

The detoxifying rate is dependent on the level of enzyme rhodanese in liver and muscles. Aminlari and Gilanpour (1991) reported that dog tissues and especially liver contained much less rhodanese activity than the same tissues of camel, horse, donkey, cattle and sheep, with brain tissue being the only exception. As a carnivorous species, the dog is not usually exposed to cyanogenic plants, hence there is little rhodanese activity in liver. The same applies to the cat. Using the same logic, it is to be expected that man is not as sensitive as the dog because man is omnivorous. The difference in sensitivity between dogs and humans was previously reported by Barcroft (1931), who exposed himself to HCN while being accompanied by a dog. The dog lost consciousness within in a few minutes, but Barcroft remained conscious and left the room without any assistance one minute after the dog had lost consciousness.

On the basis of this information it seems reasonable, as Barcroft did in his 1931 paper, to consider the goat and the monkey as one group of animals representative for humans. The following LC_{50} and LC_{01} values, estimated following Appendix F from the combined goat and monkey data of Barcroft (1931), are therefore considered to be representative for man (Table 42).

Table 42: Estimated LC values for different exposure duration (ten Berge, 2003a, 2006)

Duration (min)	LC_{50} (mg/m^3)	LC_{01} (mg/m^3)
5	676 (536 - 833)	296 (106 - 415)
10	483 (411 - 569)	211 (80 - 288)
15	396 (345 - 465)	173 (67 - 234)
20	345 (300 - 408)	151 (60 - 202)
25	309 (267 - 372)	135 (54 - 181)
30	283 (243 - 346)	124 (50 - 166)
60	202 (164 - 267)	88 (36 - 120)
120	144 (108 - 214)	63 (26 - 88)
240	103 (71 - 170)	45 (18 - 66)
480	73 (47 - 136)	32 (13 - 50)

The LC_{50} estimates fit well with reported accidental and intentional exposure (Section 9.1) (Barcroft, 1931; Bonsall, 1984).

8.1.6 Other routes

Ballantyne (1983) instilled aqueous solutions of cyanides (instilled volume 0.03 ml) in the rabbit eye and estimated the LD_{50} of the cyanides by ocular instillation (Table 43).

Table 43: Ocular toxicity

Compound	LD_{50} (mg/kgbw)	LD_{50} (mmol/kgbw)
HCN	1.05	0.039
NaCN	5.05	0.103
KCN	7.86	0.121

Experimental data from other routes like intraperitoneal (*i.p.*), *i.v.* and/or subcutaneous (*s.c.*) application have been reported (Ballantyne, 1987a). Comparison between cyanides has been considered on a mmol/kgbw basis. The *i.p.* LD_{50} of cyanides is comparable to, but usually slightly higher than the oral LD_{50} (Table 35) because of the first-pass effect of the liver by the oral route. The *i.v.* LD_{50} is more or less equal to the dose retained by inhalation in 5 minutes. The intramuscular LD_{50} is about half the oral LD_{50}. The *s.c.* LD_{50} is 1.5 to 2.5 times the oral LD_{50} probably due to the lower resorption rate via this route and the concomitant impact of detoxification. Notwithstanding these comments, it is judged that these exposure routes are not relevant for human accidental or occupational exposure.

8.1.7 Conclusion

HCN and its alkali salts are very toxic compounds by all routes of exposure. ACH is not stable at pH ≥ 7 and readily releases HCN after absorption via the oral or dermal route. The risk of poisoning by inhalation of vapours released from liquid ACH is less than that from liquid HCN because of the much lower vapour pressure of ACH.

In deriving an AEGL for HCN, the US-NAC (2000a) considered the studies of Lapin and Mackay (1981) and Ballantyne (1994a,b). The earlier study by Barcroft (1931) was quoted, but only in regard to qualitative information on interspecies variability. The ECETOC Task Force used the data of the three studies for the same test species and evaluated the concentration-time response relationships. The results for rats and rabbits were fully comparable between all studies. So the later papers confirmed the observations of Barcroft (1931) for rats and rabbits. The Task Force concluded that the observation of HCN toxicity in other species as reported by Barcroft (1931) is also of sufficient reliability to be used for estimation of the variability of the interspecies sensitivity. According to Barcroft (1931) and McNamara (1976) the sensitivity of humans is comparable to that of monkeys and goats. On this basis, the Task Force combined the observations on goats and monkeys into one group. The LC_{50} and the LC_{01} values of this combined group were estimated. These values are believed be a good estimate of the sensitivity and variability of the acute inhalation toxicity of HCN to humans.

8.2 Skin, respiratory tract and eye irritation, sensitisation

Free HCN has a pKa of 9.11 at 30°C (Table 1) and the expected pH of an aqueous solution is approximately 4 to 5. This pH is not expected to elicit an irritant reaction of the skin or of the eye. The aqueous solutions of alkali salts of cyanide, in contrast, have a pH of approximately 11 to 12, and may therefore be expected to be overtly corrosive to eyes and perhaps skin.

The amount of test material required in a standard skin or eye irritation study (500 and 100 mg/animal, respectively) is considerably above the level of systemic toxicity of cyanide. For example, for KCN, the dermal LD_{50} is 22.3 mg/kgbw (Table 36) and the ocular toxicity 7.86 mg/kgbw (Table 43).

No specific animal data on skin sensitisation are available.

Kolpakov and Prohorenkov (1978) applied two drops of NaCN (1.7 mg/kgbw) to the skin of rabbits. Skin sensitisation was determined in terms of neutrophil damage, leukocyte alteration/agglomeration and basophil degeneration. The authors concluded that NaCN had a marked sensitising effect on rabbit skin.

Human data are given in Section 9.1.2.

8.2.1 Summary

The systemic toxicity of cyanides is sufficiently high that systemic intoxication and death are likely to occur before the development of local inflammatory irritation effects on the skin or in the eyes.

8.3 Repeated dose toxicity

A number of repeated-dose toxicity studies with administration via the oral or inhalation route have been conducted in different animal species. Repeated-dose dermal studies are not available. Only a few guideline studies are available and many of the oral studies were related to the examination of the effects of cassava diets. Of the latter, only those that also examined a cyanide-treated group of animals were included. In studies employing a bolus type dosing regime, effects can frequently be attributed to acute peaks of systemic poisoning resulting from the rapid absorption profile and the steep dose response curve for acute toxicity (Appendix G). While feeding studies tend to ensure a more gradual absorption profile they often suffer from insufficient standardisation of the diet with regard to factors that will influence thiocyanate

related effects on the thyroid e.g. iodine and protein content. In many of the cassava studies the objective was to study possible effects of diets containing cyanogenic glucosides as a main source of nutrition on human health in an animal model, or to assess their effects when used as an animal food. Although these studies may provide some insight into the mode of action of repeated cyanide toxicity, they are of limited value for the derivation of a classical NOAEL.

Considering the above mentioned limitations, only a few studies may be regarded as key for the evaluation of repeated cyanide toxicity. These are the drinking-water study in rats and mice (Hébert, 1993) and, with some restrictions, the dietary studies in rats by Philbrick *et al* (1979) and Kreutler *et al* (1978), and the feeding study in pigs by Tewe and Maner (1980) (Section 8.3.1). For inhalation, the two guideline studies conducted with ACH in rats (Monsanto, 1981d, 1984) are relevant for cyanide as well (Section 8.3.3).

8.3.1 Oral

Details and results of the available repeated oral dose studies are presented in Table 44.

Table 44: Repeated oral toxicity

Species (route)/ Strain, number and sex/group	Test substance	Dose (mg/kgbw/d)	Dose (mg CN^-/ kgbw/d) [a]	Frequency of treatment	Duration	Result	Remark	Reference	CoR
Rat (drinking water)									
F344, 10 M	NaCN	0	0	*Ad libitum*	13 wk	No mortality. 12.5 mg CN^-/kgbw M: slight ↓ final mean bw and bw gains. At 12.5 and 4 - 4.3 mg CN^-/kgbw: ↓ water consumption. No biologically significant changes in haematology, clinical chemistry or urinalysis apart from ↑ urinary thiocyanate excretion. No exposure-related gross or microscopic changes in any of the organs.		Hébert, 1993	1a
		0.3	0.16						
		0.9	0.48						
		2.7	1.4						
		8.5	4.5						
		23.6 [b]	12.5						
10 F		0	0						
		0.3	0.16						
		1	0.53			M: dose-dependent significant ↓ caudal epididymal weight; significant ↓ epididymal and testis weights, ↓ number of spermatid heads (12.5 mg CN^-/kgbw). Marginally ↓ sperm motility in all exposed groups. F prolonged pro-oestrus and di-oestrus, shortened metoestrus and oestrus			
		3.2	1.7						
		8.2	4.3						
		23.5 [b]	12.5						

Table 44: Repeated oral toxicity (cont'd)

Species (route)/ Strain, number and sex/group	Test substance	Dose (mg/kgbw/d)	Dose (mg CN^-/ kgbw/d) [a]	Frequency of treatment	Duration	Result	Remark	Reference	CoR
Rat (drinking water) (cont'd)									
Sprague-Dawley, ≈ 30 M	KCN	0 40 80 160/140	0 16 32 64/56	*Ad libitum*	13 wk	Dose-related ↓ water consumption, ↓ food consumption, dose-dependent ↑ in urinary protein concentration. In the high-dose group several changes in absolute organ weights. Dose-related ↑ in relative organ weights. ↓ thymus weight in high-dose group. Histopathology: no treatment-related changes in brain, heart, liver, kidney, testes, or thyroid	Mortality at 64 mg CN^-/kgbw, and dose was ↓. Leuschner *et al*, 1991 was a satellite study	Leuschner *et al*, 1989a cited by WHO, 1993	4b
Wistar, 6 M	KCN	0 9 12	0 3.6 4.8	*Ad libitum*	15 d	Dose-related ↑ in plasma SCN^-, ↓ bw gain, no changes in blood glucose or pancreas histopathology	Examination limited to bw, blood glucose or pancreas	Soto-Blanco *et al*, 2001a	2e

Table 44: Repeated oral toxicity (cont'd)

Species (route)/ Strain, number and sex/group	Test substance	Dose (mg/kgbw/d)	Dose (mg CN^-/ kgbw/d) [a]	Frequency of treatment	Duration	Result	Remark	Reference	CoR
Rat (drinking water) (cont'd)									
Wistar, 6 - 10 M	KCN	0 0.3 0.9 3 9	0 0.12 0.36 1.2 3.6	*Ad libitum*	15 d	Dose-related ↑ in plasma SCN^-, but less at high-dose group. No changes in T3 and T4 levels. AST ↑ at 0.12, 0.36 and 1.2 mg CN^-/kgbw, but ↓ at high dose. No treatment-related changes in ALT, creatinine, urea plasma levels. At 1.2 and 3.6 mg/kgbw: congestion and vacuolisation of kidney proximal epithelial cells. At 3.6 mg/kgbw hydropic degeneration of liver cells. Dose-dependent ↑ of vacuolisation of thyroid, but no indication of hyperplasia (mild findings also in controls)	Limited examination. Diet composition not stated. Effects could be related to diet rather than treatment	Sousa *et al*, 2002	3b

Table 44: Repeated oral toxicity (cont'd)

Species (route)/ Strain, number and sex/group	Test substance	Dose (mg/kgbw/d)	Dose (mg CN^-/ kgbw/d) [a]	Frequency of treatment	Duration	Result	Remark	Reference	CoR
Rat (diet)									
Sprague-Dawley, 8 (sex not stated)	KCN	0 100 [c]	0 40	*Ad libitum*	56 d	↑ blood and urine thiocyanate levels. No signs of intoxication: NOAEL > 40 mg CN^-/kgbw/d. Significant ↑ of serum thiocyanate concentration, ↓ by protein deficiency. Liver rhodanese activity not influenced by protein deficiency, but kidney rhodanese activity ↓	Limited examinations. Diet with different composition	Tewe and Maner, 1982	2e
Albino (strain not stated), 10 F	KCN	0 2,500 5,000 [d]	0 1,000 2,000	*Ad libitum*	14 wk	↓ bw. ↓ of haemoglobin, packed cell volume, total serum protein and serum T4. ↓ relative spleen weight, ↑ relative thyroid weight, centrilobular liver cell necrosis. Benign tumours in caecum and colon: 5/10 rats at mid-dose and 7/10 at high-dose. LOEL 1,000 mg CN^-/kgbw	Reported doses exceed levels of acute toxicity by 2 orders of magnitude	Olusi *et al*, 1979	3b
Sherman, 110 (sex not stated)	KCN	250	100	*Ad libitum*	90 d	No mortality, no other data given	Very limited reporting	Hayes, 1967	3a

Table 44: Repeated oral toxicity (cont'd)

Species (route)/ Strain, number and sex/group	Test substance	Dose (mg/kgbw/d)	Dose (mg CN^-/ kgbw/d) [a]	Frequency of treatment	Duration	Result	Remark	Reference	CoR
Rat (diet) (cont'd)									
Not stated, 10 M weanling	KCN	0 99 or 135 [e]	0 40 or 54	*Ad libitum*	11.5 months	↓ bw only after 4 months. Plasma T4 level and T4 secretion ↓. Myelin degeneration in spinal cord white matter in rats on restricted diet aggravated by KCN	Diet normal (10% casein with added iodine, vitamin B_{12} and DL-methionine) or restricted (without iodine, vitamin B_{12} and ↓ DL-methionine supplement)	Philbrick *et al*, 1979	3b
Wistar, 20 M initially 4 wk of age	Not stated	-	0, 0.18 [f]	*Ad libitum*	12 months, interim kill at 3, 6 and 9 months	At 12 month only slight congestion of cerebral blood vessels	Limited examination, effects reported very briefly. Artefact possible. Aim of study to investigate different cassava ingredients alone and in combination	Ezeanyika *et al*, 1999	3b

Table 44: Repeated oral toxicity (cont'd)

Species (route)/ Strain, number and sex/group	Test substance	Dose (mg/kgbw/d)	Dose (mg CN^-/ kgbw/d) [a]	Frequency of treatment	Duration	Result	Remark	Reference	CoR
Rat (diet) (cont'd)									
Holtzman, 10 - 24 M weanling	KCN	140	56	7 g food/d	14 d	Only in iodine deficient animals: thyroid weights ↑, TSH levels ↑	Limited investigation targeted on thyroid effects. Different diets, iodine and/or protein-deficient	Kreutler *et al*, 1978	2e
Rat (gavage)									
Wistar, 6 - 7 M weanling	KCN	0 0.15 0.3 0.6	0 0.06 0.12 0.24	1 ×/d	3 months	No deaths, no clinical signs of toxicity. No effects on bw gain or food consumption. No effects on plasma glucose, T3 or T4 levels. Plasma cholesterol levels significantly ↓ at 0.24 mg CN^-/kgbw. No histopathological changes in thyroid or pancreas. Dose related ↑ in spheroids in spinal cord in all dose groups. Neuronal loss in hippocampus more intense at 0.24 mg/kgbw	Limited examination: Blood glucose, T3, T4 levels, cholesterol, histopathology only of pancreas, thyroid and CNS. Significance of findings unclear	Soto-Blanco *et al*, 2002	3b

Table 44: Repeated oral toxicity (cont'd)

Species (route)/ Strain, number and sex/group	Test substance	Dose (mg/kgbw/d)	Dose (mg CN^-/ kgbw/d) [a]	Frequency of treatment	Duration	Result	Remark	Reference	CoR
Mouse (drinking water)									
B6C3F_1, 10 M	NaCN	0	0	*Ad libitum*	13 wk	No mortality. F at 28.8 mg CN^-/kgbw: slight ↓ final bw. M/F at 24.3/28.8 and 8.6/9.6 mg CN^-/kgbw: ↓ water consumption. No biologically significant changes in haematology, clinical chemistry or urinalysis apart from ↑ urinary thiocyanate excretion. No exposure related gross or microscopic changes in any of the organs. M at 24.3 mg CN^-/kgbw: slight ↓ epididymal and caudal epididymal weights. No other effects related to reproduction toxicity. NOAEL in M/F 8.6/9.6 mg CN^-/kgbw/d		Hébert, 1993	1a
		0.5	0.27						
		1.8	0.95						
		5.1	2.7						
		16.2	8.6						
		45.9 [b]	24.3						
10 F		0	0						
		0.6	0.3						
		2.1	1.1						
		6.2	3.3						
		19.1	9.6						
		54.3 [b]	28.8						

Table 44: Repeated oral toxicity (cont'd)

Species (route)/ Strain, number and sex/group	Test substance	Dose (mg/kgbw/d)	Dose (mg CN^-/ kgbw/d) [a]	Frequency of treatment	Duration	Result	Remark	Reference	CoR
Rabbit (diet)									
New Zealand White, 6 M	KCN	0 42.8 - 60.25 [g]	0 17.1 - 24.1	*Ad libitum*	40 wk	↑ food consumption and ↓ bw gain. Significant ↑ in serum creatinine, urea, urinary thiocyanate, ↑ in lactate dehydrogenase activities in liver, kidney and serum. Sorbitol dehydrogenase and glutamate-pyruvate transaminase in liver ↓, in serum ↑, alkaline phosphatase ↓ in liver and kidney, rhodanese activity ↑ in serum, liver and kidney. Histopathological changes in liver and kidney. No other organs examined		Okolie and Osagie, 1999	2e
New Zealand White, 6 M	KCN	0, 87 [h]	34.8	2 ×/d	6 wk	↑ food consumption. No change in bw gain. ↓ activities of Na-K-ATPase, Ca- and Mg-ATPase levels in ileum, colon, liver, kidney	Limited investigation, methods not well described	Okolie *et al*, 1994	3a

Table 44: Repeated oral toxicity (cont'd)

Species (route)/ Strain, number and sex/group	Test substance	Dose (mg/kgbw/d)	Dose (mg CN^-/ kgbw/d) [a]	Frequency of treatment	Duration	Result	Remark	Reference	CoR
Dog (feeding)									
Not stated, 6 M 5 months of age	NaCN	1.76 [i]	0.93	1 ×/d	14 wk	No clinical symptoms related to treatment observed. ↓ bw gain. ↑ plasma thiocyanate levels after 1, 3, 14 wk. No effects on plasma lipase levels compared to controls. ↓ serum alanine, ↑ serum leucine, isoleucine, valine and 3-methylhistidine. Histopathology of pancreas: necrosis, fibrosis and atrophy of acinar tissue. No significant effects on growth rate, muscular mass or bone development. No clinical symptoms related to treatment T3 levels significantly ↓ in wk 14 only compared to controls. ↑ serum phenyl-alanine and tyrosine levels. Thyroid weights ↑, but not significant. Microscopy of thyroid: thickened walls of follicles, only few follicles with colloid, pale staining, slightly basophilic, desquamation of epithelial cells to lumina of empty follicles	Clinical chemistry limited to thiocyanate levels, plasma free amino acids, plasma lipase and T3. Histopathology of pancreas and thyroid only	Kamalu, 1991; Kamalu and Agharanya, 1991; Ibebunjo *et al*, 1992	2e

Table 44: Repeated oral toxicity (cont'd)

Species (route)/ Strain, number and sex/group	Test substance	Dose (mg/kgbw/ d)	Dose (mg CN^-/ kgbw/d) [a]	Frequency of treatment	Duration	Result	Remark	Reference	CoR
Dog (feeding) (cont'd)									
Not stated, 6 (sex not stated)	NaCN	1.76	0.93 [i]	1 ×/d	14 wk	No clinical symptoms reported. ↑ plasma and urinary thiocyanate levels. Wk 3: serum albumin ↑, serum globulin ↓. Wk 14: serum albumin ↓, serum globulin normal, plasma free amino acids ↑. Kidney: occasional desquamation of tubular cells. Testes: ↓ frequency of stage 8 seminiferous tubules, abnormal cells, sloughing of germ cells. Adrenals: ↑ width of *zona glomerulosa*, hyperplasia and hypertrophy of aldosterone-secreting cells	Limited biochemistry and histopathology. Organs examined: liver, kidney, myocardium, testis and adrenal gland	Kamalu, 1993	2e

Table 44: Repeated oral toxicity (cont'd)

Species (route)/ Strain, number and sex/group	Test substance	Dose (mg/kgbw/d)	Dose (mg CN^-/ kgbw/d) [a]	Frequency of treatment	Duration	Result	Remark	Reference	CoR
Dog (feeding) (cont'd)									
Not stated, 1 F	NaCN	0.5	0.27	1 ×/d in gelatine capsule	13.5 months	Dog 1: 0.27 mg CN^-/kgbw: repeated slight symptoms of intoxication from wk 53, death due to severe acute poisoning in wk 4. Dog 2 and 3: repeated symptoms of acute intoxication for ≈ 30 min after dosing. No further clinical signs of intoxication with regard to behaviour, bw gain, food consumption, *etc.* Dose-related ↑ in number of erythrocytes and haematocrit in month 1 - 6. Return to normal in month 7 - 8. Normal liver and kidney function. Serum-thiocyanate: initial ↑ followed by ↓ (month 2 - 5), steady state around month 5. Necropsy: hyperaemia and swelling of mucous membranes of the intestine and the brain hyperplasic nodular changes of liver parenchyma cells in dog 2. Degenerative changes in ganglion cells (cerebrum) and Purkinje cells (cerebellum). Bone marrow: ↑ erythropoiesis	One dog/group only, limited investigations	Hertting *et al*, 1960	3c
		1 for first 4 months, then 2 × 2	0.53 then 2 × 1.06		10.5 months				
		2	1.06		14.5 months				

Table 44: Repeated oral toxicity (cont'd)

Species (route)/ Strain, number and sex/group	Test substance	Dose (mg/kgbw/d)	Dose (mg CN^-/ kgbw/d) [a]	Frequency of treatment	Duration	Result	Remark	Reference	CoR
Pig (drinking water)									
Pittman-Moore, 3 M/F juvenile	KCN	0, 1, 1.75, 3 [j]	0, 0.4, 0.7, 1.2 [k]	1 ×/d prior to meal	24 wk	T3 and T4 ↓ in all treated groups, fasted glucose levels, thiocyanate levels ↑, significant in high dose. Aggression, explorative behaviour ↓, distractibility from eating, limping, stiffness, anaesthesia recovery, vomiting, convulsions, ↑ shivering	Special investigation. Drinking water applied with a syringe into the mouth	Jackson, 1988a	2e

[a] Converted values (KCN × 0.4, NaCN × 0.53)
[b] Reported as 0, 3, 10, 30, 100, 300 mg/l water. Doses and concentrations as reported
[c] Reported as 0, 1.87 g/kg food. Converted using average bw ≈ 350 g/rat and food consumption ≈ 15 g/d
[d] Reported as 0, 50, 100 g/kg food. Converted using as default for adult rat 400 gbw and 20 g/d food consumption
[e] Reported as 0, 1,500 mg/kg food. Converted using average bw of 227 g (normal diet) or 167 g (restricted diet), respectively, and default food consumption of 15 g/d (young rat)
[f] Reported as 0, 1.8 mg/100 g diet. Converted using reported food intake ≈ 10 g/100 gbw/d
[g] Reported as 0, 1.75 g/kg food. Converted using bw of 1.52 to 2.13 kg/rabbit and intake of 36.5 mg CN^-/animal/d, both as reported
[h] Reported as 0, 1.6 g/kg food. Converted using average bw of 1,632 g and feed intake of 88.5 g/rabbit/d, both as reported
[i] Reported as 17.6 mg NaCN (2 × 8.8 mg/ml)/kg diet, 1 × 100 g food/kgbw/d (Ibebunjo *et al*, 1992; Kamalu, 1993)
[j] Converted values (KCN/0.4)
[kl] As reported

Rat

F344 rats received up to 300 mg NaCN/l in their drinking water for 13 weeks. In addition to the normal investigations, vaginal cytology was examined 12 days prior to killing, and sperm motility was evaluated at necropsy. Water consumption was reduced in the 100 and 300 mg/l groups with corresponding decreases in urine excretion and increases in urine specific gravity. Urinary thiocyanate levels were increased in the 10 to 300 mg/l groups. A slight decrease in final body weight and body-weight gain was observed in male rats exposed to 300 mg/l NaCN, but not in females. No other treatment-related clinical signs were observed. No treatment-related changes in haematology, clinical chemistry, gross pathology or histopathology were observed. The thyroid and CNS (in particular the *corpus callosum*) were examined thoroughly for any histopathological changes. No morphological differences, compared to controls, were observed with regard to follicle size, colloid staining or follicular epithelium in the thyroid of rats receiving NaCN. In histological sections of the brain there was no sign of treatment-related degenerative changes in the *corpus callosum*. The caudal epididymal weights of all male rats were significantly lower than those of the controls. In rats receiving 300 mg NaCN/l, left epididymal and testis weights and the number of spermatid heads per testis were also lower than in the controls. Sperm motility in all exposed male rats was less than in the controls, but the changes were not considered biologically significant by the authors and were in the range of normal values reported from different laboratories. Pro-oestrus and di-oestrus times were prolonged in female rats of the 100 and 300 mg/l groups compared to controls, without a clear dose response. According to the authors these effects cannot unequivocally be attributed to cyanide exposure. No clear NOAEL can be derived for rats due to the unclear significance of the findings concerning the reproductive organs. The NOAEL for effects other than on reproductive organs was 300 mg/l or 12.5 mg CN^-/kgbw, the highest dose used in this study. The authors conclude that while assessments of thyroid hormone levels (T3, T4 or TSH) were not performed in this study, the evaluations that were done (histopathological evaluation of thyroid gland and brain) provided no evidence of thyroid or neurological effects (Hébert, 1993). The results indicate that subchronic administration of NaCN at concentrations that caused no clinical signs of toxicity or significant reductions of body-weight gain was not thyrotoxic or neurotoxic. The significance of the effects on reproductive organs is discussed in Section 8.6.

In a 13-week toxicity study, Sprague-Dawley rats were administered KCN in the drinking water at dose levels of 40, 80 and 160/140 mg KCN/kgbw/d. Additional rats received drinking water with KCN (80 mg/kgbw) and 10% alcohol. Three control groups were used: rats drinking water *ad libitum*, paired drinking (parallel to the high-dose level KCN) and drinking water with 10% ethyl alcohol. Behaviour, external appearance, body weight, food consumption (daily) and drinking water consumption (twice weekly) were recorded frequently. Extensive haematological, clinical chemical (in serum) and urine analyses were carried out in 5 animals per group in week 6 and week 13. Autopsy and macroscopy were performed after 13 weeks (≈ 20 animals/group) and

11 organs were weighed. Histopathological examination was performed in brain, kidneys, heart, liver and testes of these animals. In addition, thyroids of the control, the paired drinking control and the high-dose group (160/140 mg/kgbw/d) were examined. There was a clear indication that reduced food consumption and body weight in the KCN groups were caused by a decrease in water consumption due to decreased palatability. Urinalysis revealed a higher level of protein in the animals receiving KCN. The amounts of protein determined increased with increasing doses of KCN and decreasing drinking water consumption. Several changes in absolute organ weights were seen at 160/140 mg KCN/kgbw/d. Relative weights of organs were very slightly increased in the 40, slightly increased in the 80 and clearly increased in the 160/140 mg KCN/kgbw groups. The thymus weight was, however, reduced in the high-dose group. Histopathological examination revealed no indication of damage to the brain, heart, liver, testes, thyroids or kidneys due to treatment with KCN (Leuschner *et al*, 1989a cited by WHO, 1993). It should be noted, however, that the dose levels used are equivalent to 16, 32 and 64/56 mg CN^-/kgbw, which exceeds the detoxification capacity of the rhodanese pathway in rats (Section 7.3).

Philbrick *et al* (1979) studied the effect of KCN addition (1,500 mg/kg) to the diet of male rats (strain not specified) for 11.5 months. The normal diet contained sufficient iodine, DL-methionine and vitamin B_{12} (≈ 40 mg CN^-/kgbw/d); the restricted diet was deprived from those supplements (≈ 54 mg CN^-/kgbw/d). Investigations were limited to determination of body and selected organ weights, plasma T4 and urinary T4 excretion rate, and histopathology of brain, optic and sciatic nerves, spinal cords and thyroids. No mortality and clinical signs attributable to cyanide treatment were observed. A decrease in body-weight gain was seen in the animals receiving normal diets with cyanide that was aggravated in animals with the restricted diet (food consumption was not reported). Plasma T4 and excreted T4 levels were significantly decreased in rats receiving KCN after 4 months, but not after 11.5 months. Significant increases in relative thyroid weight were observed in both KCN treated groups compared to controls at the end of the study. No histopathological changes in optic and sciatic nerve, neuronal tissues or thyroid were observed in the cyanide-treated animals. In the rats fed on restricted diet, myelin degradation was seen (by electron microscopy) in the spinal cord white matter. This was aggravated when the diet contained additional KCN. According to the authors, those results have to be treated with caution as considerable autolysis had occurred in the tissues of the dietary restricted animals.

A similar experiment was reported in which male and female with rats (strain not stated) received different diets, including a basal diet with 2,400 mg KCN/kg food (≈ 300 mg CN^-/kgbw/d based on average food intake ≈ 80 g/d and bw ≈ 250 g from the study) and different diets with low and high protein content plus bittersweet cassava (*Manihot esculenta*) (a plant containing cyanogenic glucosides). The highest cyanide content of the diet as a combination of KCN and glucoside was reported to be 2,984 mg CN/kg (≈ 940 mg CN^-/kgbw/d). The exposure period was 6 months. The authors reported that blood and urinary thiocyanate levels corresponded well and increased with increasing cyanide exposure indicating an adaptation of urinary thiocyanate excretion with

increasing plasma levels. No evident histopathological lesions (details not given) or impairments of thyroid function (measurements of thyroidal radioiodine uptake 24 hours before termination of the study) were reported (Guzmán *et al*, 1977). The exact composition of the diets remains unclear from the publication and no clear conclusions can be drawn.

Kreutler *et al* (1978) studied the interactions of protein deficiency, iodine deficiency, cyanide and thiocyanate in neonatal and adult Holtzman rats. Young rats (90 to 100 gbw) were fed either a protein-deficient diet (2% casein) or a diet with sufficient protein (20% casein). The casein was analysed for its iodine content and was reported to contain 0.1 g I^-/g protein. The animals were kept for 2 weeks on low or high protein and iodine deficient diets prior to treatment with cyanide. Thereafter the different groups were dosed for another 2 weeks. KCN and propylthiouracil (an antithyroid drug) were added to the diet; the diet intake was restricted to 7 g/d and adjusted between the groups. Distilled water was offered *ad libitum*. At the end of the experiment, blood was collected by cardiac puncture and plasma TSH levels determined by radio-immunological assay. Thyroids were removed and weighed (Table 45).

Table 45: Effects of iodine deficiency, cyanide and thiocyanate in male Holtzman rats
(Kreutler *et al*, 1978)

Diet	Thyroid weight		Plasma TSH
	mg	mg/100 gbw	mU/100 ml
Control 2% casein	8.1 ± 0.6	11.0 ± 0.9	5.5 ± 1.1
2% Casein, 0.2% propylthiouracil	22.0 ± 0.8	30.9 ± 1.6	170 ± 15
2% Casein, 0.2% propylthiouracil, 0.2% KCN	20.3 ± 1.3	35.1 ± 1.2	177 ± 15
2% Casein, 0.2% KCN (140 mg/kg/d)	17.5 ± 0.7	26.7 ± 1.2	147 ± 15
2% Casein, 0.2% KCN (140 mg/k/d), 14 µg I^-/g diet	5.4 ± 0.3	8.3 ± 0.6	6.3 ± 2.0
20% Casein	11.6 ± 0.8	7.3 ± 0.5	12.5 ± 0.7
20% Casein, 0.2% propylthiouracil	44.5 ± 4.2	29.2 ± 2.2	180 ± 12
20% Casein, 0.2% KCN (140 mg/kg/d)	10.3 ± 0.5	7.1 ± 0.8	11 ± 2

All rats on the low protein diet lost weight in the same manner irrespective of treatment while rats receiving 20% protein gained weight. Relative thyroid weights were higher in the protein-deficient group while absolute thyroid weights were lower. At both protein levels, propylthiouracil treatment resulted in significant increases in thyroid weight and plasma TSH levels. Rats receiving 0.2% KCN in protein and iodine deficient diets showed significant increases in absolute and relative thyroid weights and plasma TSH compared to the respective

controls. Addition of propylthiouracil to a low protein, low iodine and KCN diet resulted in an aggravation of the effects. Addition of iodine to the protein-deficient KCN diets prevented the effects on thyroid and TSH completely. Rats fed a 20% protein diet and 0.2% KCN showed no effects on the thyroid weight and little or no effect on TSH. The authors explained the lack of effects on the thyroid in the high protein group as being due to the animals receiving sufficient iodine via the protein (≈ 0.14 µg I^-/d).

In a second experiment, Kreutler *et al* (1978) administered thiocyanate in concentrations of 0, 4, 8 or 16 mg SCN^-/100 ml in the drinking water of 1- to 2-days pregnant Charles River rats (5 or 7/group) receiving either Remington low iodine diet or normal rat chow. Drinking water consumption was approximately 40 ml/d. At birth each litter was adjusted to 6 pups. 24 hours before final kill of the mothers they received *i.p.* injections of radiolabelled thiocyanate ($^{35}SCN^-$, 20 - 40 µCi/rat). Milk was obtained by manual expression, maternal blood by cardiac puncture and both analysed for $^{35}SCN^-$ using paper chromatography-scintillation counting. The blood of the pups was analysed in the same manner. Thyroids of mothers and pups were isolated and weighed. Plasma thiocyanate levels were higher in control rats receiving normal rat chow than in those on the iodine deficient diet. The plasma levels reflected the exogenous thiocyanate levels contained in the diet. Plasma thiocyanate levels in mother rats on Remington low iodine/low thiocyanate diet receiving thiocyanate in their drinking water increased in proportion to the dose. Plasma levels in pups also increased, but less than in the mothers. The highest level of thiocyanate intake (16 mg SCN^-/100 ml) resulted in increased thyroid weights. The thiocyanate intake corresponded to plasma levels of 1,526 ± 100 µg SCN^-/100 ml plasma at day 5 *post partum* and 1,280 ± 127 µg SCN^-/100 ml plasma at day 10 *post partum* in the dams. In the pups, increased thyroid weights were also observed in a dose related manner already at maternal dose levels of 8 mg/100 ml at day 10 *post partum*. The effects were observed at pup-serum thiocyanate levels of 279 to 333 µg/100 ml. The thiocyanate blood levels were in the range of those of the pups receiving normal rat chow. The combination of iodine deficiency and elevated thiocyanate blood levels in the mothers seems to have led to thyroid enlargement in the pups. The amount of thiocyanate in the milk was dependent on the plasma levels of the mother animals. However, with increasing exogenous thiocyanate, more of it was excreted in the urine. The authors confirmed the latter information by studying the disposal of stable and $^{35}SCN^-$ in adult male rats. Independent of iodine intake, plasma and urine concentrations were increased in rats receiving SCN. Renal excretion of $^{35}SCN^-$ was sharply increased in rats receiving thiocyanate in their drinking water.

The effects of repeated oral (gavage) doses of KCN (0.06 - 0.24 mg CN^-/kgbw) on the CNS, thyroid and pancreatic metabolism were studied in male Wistar rats exposed for 3 months. The dose levels approximated to 1.5, 3 and 5% of the oral LD_{50}. The animals had free access to a standard rat diet and water during the study. At the end of the study plasma cholesterol, glucose, T3 and T4 levels were measured in blood samples taken from the hepatic vein under ether

anaesthesia. Histopathology of CNS, thyroid glands and pancreas was performed after formalin perfusion. CNS and thyroid gland fragments were embedded in paraffin and micrometer slices were stained with haematoxylin-eosin. Food consumption, body-weight development and clinical signs were comparable to controls in all experimental groups. No treatment-related differences in plasma glucose, T3 or T4 levels between treated and control groups were observed. Plasma cholesterol levels in the 0.24 mg/kg cyanide group were significantly lower than those of controls. Histopathological examination of the pancreas and thyroid did not reveal any pathological changes in any of the groups. Several findings in the differential CNS sections were attributed to cyanide treatment by the authors. Neuronal loss in the hippocampus was reported to be more intense in animals of the high-dose group. Spheroids in the white matter of the spinal cord were reported in all experimental groups and a dose-related increase in number was reported. In animals of the higher dose groups (not specified by the authors) damage of Purkinje cells and loss of white matter was reported in the cerebellum (Soto-Blanco *et al*, 2002). The histopathological lesions are, however only briefly reported and it is possible that, given the low number of animals per group, the findings could also be related to the processing of the sections and slides.

A drinking-water study was conducted in male Wistar rats receiving 0, 0.3, 0.9, 3 or 9 mg KCN/kgbw/d for 15 days. The composition of the diet was not given in the paper. At the end of the experiment blood samples were taken and blood thiocyanate levels as well as thyroid hormone levels (T3 and T4), liver enzymes, creatinine and urea were determined. Histopathology was performed on kidneys, liver (left lobe) and thyroid. Water consumption was not affected by the treatment: food consumption was not measured. A significant reduction in weight gain was observed in the highest dose group compared to controls. Plasma thiocyanate levels were increased in all dose groups, but no effects were observed on T3 or T4 levels. Plasma levels of alanine aminotransferase (ALT) were changed in some dose groups, but showed no dose relationship, while plasma aspartate aminotransferase (AST) levels increased in the first 3 dose groups, but decreased at the highest dose: creatinine and urea levels were not different from controls. The authors report histological changes in the kidney of the 3 and 9 mg/kgbw groups that consisted of congestion, cytoplasmic vacuolisation of the epithelial cells of the proximal tubules. In the livers of the 9 mg/kg dose group hydropic degeneration was observed (vacuoles, periodic acid Schiff and Sudan staining negative). The authors claimed to have observed a dose-dependent increase in the number of re-absorption vacuoles in the thyroid of all dose groups and to a minor extent in the controls, but no follicular cell hyperplasia. As the composition of the diet, in particular the iodine and protein content was not given, the latter finding remains questionable and could be related to deficiencies in the diet. Protein deficiency causes follicular atrophy and the observed effects could be an early sign. The effects on body-weight gain and the relatively low thiocyanate levels in the 9 mg/kgbw group also point in the direction of protein deficiency. Liver and kidney histopathology findings were also not in line with the clinical chemical observations (at 3 and 9 g/kgbw, one would have expected increased AST, ALT, creatinine and

urea levels in blood) and could perhaps also be related to the experimental techniques or dietary influences (Sousa *et al,* 2002). No comparable findings in liver, kidneys or thyroids were reported in the 90 day study of Hébert *et al* even though higher cyanide dose levels were used. The relevance of the findings in this 14-day study with a limited number of animals thus remains questionable and could be related to dietary deficiencies rather than to cyanide exposure alone.

To study possible diabetogenic effects of cyanide, male Wistar rats received daily doses of 0, 9 or 12 mg KCN/kgbw in their drinking water for 15 days. The animals were fed a standard rat chow. Venous blood samples were taken at the end of the experiment from the hepatic vein and were analysed for thiocyanate and glucose. Three animals of each group were necropsied at the end of the study and the pancreas was examined histopathologically. Body-weight gains of exposed rats were lower than that of controls. Thiocyanate plasma levels showed a dose-dependent increase. No effects were observed on the glucose levels and no gross or histopathological changes were observed in the pancreas (Soto-Blanco *et al*, 2001a).

In a study comparing cassava diets with normal diets, female albino rats were fed 0, 10 or 50 g KCN/kg food (≈ 1,000 or 2,000 mg CN^-/kgbw/d) for 14 weeks. Food consumption was not reported. A dose related decrease in body weight was observed (7.25 and 15%) in the cyanide-exposed rats, while control rats on normal diet gained weight (10%). Haemoglobin levels, packed blood cell volumes and total serum proteins were lower in the cyanide groups compared to controls. Serum thiocyanate levels showed a dose related increase compared to controls. The authors also report a dose-related decrease in T4 levels compared to controls. Organ-weight determinations were restricted to the thyroid and the spleen. Relative thyroid weights showed a dose-related increase in the cyanide groups. Relative spleen weights were only decreased in the high-dose cyanide group. Histopathological changes in the thyroid of these groups were not reported in the publication. In the liver of the cyanide-treated animals, centrilobular liver cell necrosis was observed. Five of 10 rats on the 50 g/kg diet and 7 of 10 rats on the 10 g/kg diet were reported to have tumours of the caecum and colon that consisted of hypertrophic muscle fibres with no evidence of a malignant change (Olusi *et al*, 1979). The study is questionable because the reported doses exceed the levels of acute toxicity by about two orders of magnitude. Survival can only be explained by a significantly reduced food intake that would, in part, explain the pathological findings.

Ezeanyika *et al* (1999) studied the effects of scopoletin (an ingredient of cassava) and cyanide on rat brain histopathology in Wistar rats that were fed diets supplemented with vitamins and iodine (in the same mix, which could have led to oxidation of I^-) (exact composition given in the paper) for 12 months. The treatment groups received either 0.07 mg/100 g diet of scopoletin, 1.8 mg/100 g diet of cyanide or both 0.07 mg scopoletin and 1.8 mg CN^-/100 mg diet. The animals received food and water *ad libitum*. Interim kills were performed after 3, 6 and 9 months. Relative brain weights, brain histopathology and determination of lipid peroxidation products in the brain were

performed. Treatments had no effect on food intake, but body-weight gain was significantly reduced in the scopoletin and cyanide treatment groups, but not in the group receiving combined scopoletin and cyanide exposure. Brain weights were significantly reduced compared to controls in the scopoletin/cyanide group only from 6 months. None of the treatments had effects on lipid peroxidation in the brain. Congestion of the meninges and cerebral blood vessels with perivascular oedema and neuronal degeneration and necrosis were observed in the scopoletin and scopoletin/cyanide groups from 6 months. In the combined group, additional changes such as haemorrhage and mononuclear infiltration were observed. The severity increased considerably with the exposure period. In the cyanide group, only slight vascular changes were observed that were manifest only at 12 months of exposure (no details given).

To investigate a possible effect of the lack of sulphur-amino acids (SAAs) on neurological effects (tropical neuropathy 'konzo') related to cassava rich diets in parts of Africa, female Sprague-Dawley rats (16/group) were fed a balanced diet containing sufficient amounts of all essential amino acids or a diet free of sulphur-containing amino acids. All rats received an equivalent of 50 mg/kgbw of KCN in their drinking water for up to 4 weeks. Groups of 4 animals of each group were killed after 1, 2, 3 and 4 weeks. Before treatment the rats were kept for 7 days on standard rat chow and baseline values of thiocyanate and inorganic sulphate in the 24-hour urine at day 7 were determined. Intracardiac blood samples were taken from 4 animals at the beginning of the study and baseline cyanide and cyanate levels were determined. Prior to the respective termination times, 24-hour urine samples were analysed for thiocyanate and inorganic sulphate and cardiac blood samples for cyanide and cyanate. Food intake in animals fed SAA-free diets was significantly less than in rats receiving the basal diet. Rats on the SAA-free diet had a "general weakness", were hyper-responsive to noise, and excreted a brownish red material around their necks (porphyrins). The total water and KCN intake was similar between the groups. Rats on SAA-free diet lost body weight during the study while rats fed basal diet gained weight. Urinary output of inorganic sulphate had more than doubled in rats fed basal diet after 1 week and remained constant thereafter, while in SAA-deficient animals inorganic sulphate in urine dropped to below detection limits in week 1 and stayed undetectable thereafter. Average urinary thiocyanate levels were about 5 times elevated compared to base levels in each group at every time point. Blood cyanide levels increased at a rate of approximately 29.4 µmol/l/wk and developed in a comparable matter in both groups. Blood cyanate levels in SAA-deficient rats were significantly higher in weeks 3 and 4 compared to the basal diet group. Both treatment groups had higher plasma cyanate levels than control groups on normal rat chow not exposed to cyanide in drinking water. The authors concluded that elevated cyanate blood levels might contribute to neurological diseases related to a deficiency in SAAs and elevated exposure to cyanide (Tor-Agbidye *et al*, 1999).

Mouse

$B6C3F_1$ mice received doses of up to 300 mg NaCN/l in their drinking water for 13 weeks. In addition to the normal investigations, vaginal cytology was examined 12 days prior to killing and sperm motility was evaluated at necropsy. Water consumption was reduced in the 100 and 300 mg/l groups. A slight decrease in final mean body weight was seen in female mice exposed to 300 mg NaCN/l, but not in the males. No other treatment-related clinical signs were observed. No treatment-related changes in haematology, clinical chemistry, gross pathology or histopathology were observed. The thyroid and CNS were examined thoroughly for any histopathological changes. The caudal epididymal weights of the 300 mg/l male mice were significantly lower than those of the controls. No changes in sperm motility were observed. No prolongation of pro-oestrus or di-oestrus times occurred in the females Thus a NOAEL of 100 mg/l or 8.6 to 9.6 mg CN^-/kgbw/d could be derived from this study. The NOAEL for effects other than on reproductive organs was 25.6 mg CN^-/kgbw, the highest dose applied in this study. The authors concluded that while assessments of thyroid hormone levels (T3, T4 or TSH) were not performed in this study, the evaluations that were performed (histopathological evaluation of thyroid and brain) provided no evidence of thyroid or neurological effects (Hébert, 1993). The results indicate that subchronic administration of NaCN, at concentrations that caused no clinical signs of toxicity or significant reductions of final body weight, was not thyrotoxic or neurotoxic. The significance of the effects on reproductive organs is discussed in Section 8.6.

Rabbit

To study the effects of chronically high dietary levels of KCN on liver and kidney, New Zealand White rabbits received about 20 mg CN^-/kg/d in their diet for 40 weeks. Body-weight gain was reduced when compared to untreated controls, despite a higher food intake. Urinary thiocyanate, serum urea and creatinine levels were significantly higher in treated than in control animals. Lactate dehydrogenase and rhodanese activities were higher in kidney, liver and serum of treated animals compared to controls. There was a reduction of sorbitol dehydrogenase, glutamate pyruvate transaminase in liver and alkaline phosphatase activities in liver and kidney with a corresponding increase of those enzyme activities in serum of the cyanide-treated animals compared to controls. Histopathological findings in liver and kidney consisted of congestion and necrosis in the liver and tubular and glomerular necrosis in the kidney (Okolie and Osagie, 1999).

Dog

Kamalu and Agharanya compared the effects of dried cassava (gari) diets with cyanide-enriched diets in growing dogs over a 14 week period. The results of this single study were reported in four

separate publications (Kamalu, 1991, 1993; Kamalu and Agharanya, 1991; Ibebunjo *et al*, 1992). The dogs used in the studies were purchased at the local markets. The cassava and enriched cyanide diets contained equivalent amounts of cyanide (≈ 0.93 mg CN^-/kgbw/d) and were administered with 500 g food/d (≈ 100 g food/kgbw/d). The carbohydrate source in the diet was rice. The diet contained lean pork (130 g pork/kg dry food of the control and cyanide groups and 210 g/kg dry food in the cassava group) and was supplemented with vitamins and minerals, including 19.6 mg KI/kg diet (9.8 mg KI/d based on 500 g diet/d). The additional meat received by the cassava fed dogs was acknowledged by the authors to be another possible source of iodine for this group (Kamalu and Agharanya, 1991).

In both papers of 1991, the dogs receiving cyanide were reported to have gained less weight than the other groups, but quantitative data on body weight were not reported. Kamalu (1991) reported increased plasma thiocyanate levels in dogs fed cyanide-enriched diets (≈ 0.93 mg CN^-/kgbw/d, 14 wk) and to a lesser extent in cassava fed animals. Thiocyanate plasma levels in the controls were set to zero (by the authors) and used as "thiocyanate free standards" for the measurement of the thiocyanate levels in the cyanide or cassava fed animals (Kamalu, 1993). This means that variations in background levels of plasma thiocyanate were not considered by the authors. Serum lipase levels were not elevated in the cyanide-treated groups, but were in the cassava groups indicating more severe effects of cassava on the pancreas. Histopathology of the pancreas, the only organ reported in this paper, showed fibrous degeneration of Langerhans glands as well as necrosis and atrophy of the acinar tissue. All effects on the pancreas, except fibrosis, were more pronounced in animals fed cassava compared to the cyanide-treated groups. The authors evaluated the insulin status indirectly from the levels of different amino acids in plasma (gluconeogenesis index). Low insulin secretion depresses amino acid uptake and protein synthesis and increases protein catabolism in the muscle leading to elevated blood levels of branched amino acids and an increased utilisation of alanine for glucose synthesis. The gluconeogenesis index was 5 times higher than controls in the cassava group and twice as high in the cyanide-treated group indicating hypoinsulinaemia. Quantitatively the gluconeogenesis index was neither related to thiocyanate levels or the severity of the pancreatic lesion.

Kamalu and Agharanya (1991) reported thyroid hormone (T3) levels. In control animals, T3 levels increased with time during the study, in cassava treated animals an initial decrease was followed by an increase. In the cyanide groups, the T3 levels remained essentially unchanged until week 5 but were decreased in week 14. Interestingly, T3 levels in the cassava group were elevated compared with all other groups at time zero (before treatment). The authors did not report the time of day at which samples were taken, or whether this varied between groups or time points. Thyroid weights were elevated in the cyanide-treated group at the end of the exposure period, but this increase was not statistically significant. Histopathology of the thyroid (the only organ reported in this paper) revealed indications of hyperplasia of follicular cells and increase of parenchyma in the cyanide-treated group. These changes were seen to a lesser extent

in the rice control group, but not in the cassava group. In the control and cyanide group more follicles devoid of colloid were observed than in the cassava fed groups. This is indicative of an iodine deficiency in the control and cyanide groups.

In the third paper, Kamalu (1993) reported the remainder of the histochemistry and blood chemistry data. Plasma thiocyanate levels in cyanide and cassava treated dogs increased with increased exposure time. According to the author, this accumulation was probably due to re-absorption of thiocyanate from the kidney. The author stated that thiocyanate re-absorption rates in the kidney may exceed the excretion rate. Protein was excreted in the urine of cassava fed dogs (from week 1) and cyanide-treated dogs (from week 5). Total serum protein levels were not different between the groups. No significant differences in albumin or globulin levels between the control and dose groups were observed. An increase of free amino acids in plasma was observed at the end of the 14-week treatment period in dogs fed cyanide-enriched rice diets when compared to controls. The author attributed this effect to a decreased utilisation of amino acids.

Furthermore, Kamalu (1993) observed periportal vacuolation and congestion in the liver of dogs fed cassava, but no hepatic effects were observed in dogs fed rice with NaCN added. Occasional desquamation of tubular cells in the kidneys was observed in cyanide-treated animals, while the cassava diet caused more severe kidney damage. The author analysed 1,200 testicular cross-sections from each group of dogs and identified an increased frequency of stage 8 seminiferous tubules with occasional occurrence of cells with large pyknotic nuclei and eosinophilic cytoplasm or multinucleated cytoplasm in cyanide-treated dogs only compared to controls. (Stage 8 of the spermatogenic cycle in dogs is characterised by the lining of the seminiferous tubular lumen by elongated spermatids.) Extensive sloughing of germ cells was also observed in this treatment group. (The significance of the reproductive endpoints is discussed in Section 8.6.) Effects on the adrenal gland, including swelling, haemorrhage, and fibrosis were observed in dogs fed cassava, as well as in dogs fed rice with NaCN added. In cyanide-treated dogs, the width of the *zona glomerulosa* was significantly increased compared to that of the other groups, while the adrenal medulla of the control and cyanide group appeared normal. Cassava fed dogs showed haemorrhage in the cortico-medullar region and congestion throughout the medulla accompanied by small groups of chromaffin cells with pyknotic nuclei and an increase of pyknotic tissue.

In the fourth paper, Ibebunjo *et al* (1992) reported on the development of muscle and bones under the influence of the different diets. The authors observed similar growth rates for all three groups of dogs. Although the cyanide fed dogs had a lower relative body-weight gain than the other groups (growth rate of 1.51 compared to 1.73 in controls and 1.83 for the cassava group) it was not reported to be statistically significant. The cassava fed dogs were reported to have a greater amount of *s.c.* fat than the control or cyanide groups. Cranial tibial and pectineus muscles of the cassava fed group were significantly smaller than those of the controls, while the weight difference of the *biceps bracchii* and the common digital extensor muscle between cassava and

control group animals did not reach significance. No significant differences in bone weights, length or diameters of the humerus, radius and ulna bones of the forelimbs, and the femur, tibia and fibula bones of the hind limbs were observed.

Deficiencies in the dog study

The Task Force noted several deficiencies in the dog study reported by Kamalu (1991, 1993), Kamalu and Agharanya (1991), and Ibebunjo *et al* (1992).

The first concern relates to the study design and dietary presentation. It should be acknowledged that the authors recognised that, on the basis of the divergent effects observed with rice and cyanide compared to cassava diets, the composition and nutritional adequacy of the diet is an important parameter in the design and interpretation of dietary studies. The histopathological changes in the thyroid of control animals indicate that the diet, despite having added iodine, may have been effectively iodine deficient. In contrast, and rather ironically the addition of extra meat to the diet of the cassava fed animals may have resulted in this diet being iodine enriched and perhaps even iodine sufficient.

The profile of release and absorption of cyanide from cassava and rice with cyanide is different. Cyanide is released more slowly from cassava resulting in diffusion-controlled release of thiocyanate with a lower profile of blood thiocyanate (Kamalu, 1993). In contrast, cyanide absorption from NaCN supplemented rice will be very rapid giving rise to a sharp peak in absorbed cyanide and a more sustained thiocyanate level that is mainly controlled by the metabolic formation (Vmax) at saturation concentration and its urinary elimination rate. This difference in kinetics is obvious from Figure 1 in the Kamalu (1991) paper. High peak cyanide blood levels would be unavoidably linked to an increased likelihood of overt toxicity due to free cyanide. Although food consumption was not reported, reduced body-weight gain was noted in the NaCN rice fed dogs. This was likely due to repeated acute-toxicity, which may have affected food consumption and growth of the dogs.

The second concern relates to the observed changes in thyroid hormones and their possible significance. Thyroid hormone regulation is very complex and affected by many parameters, many of which can be perturbed unintentionally in a study of this design. The authors used dogs of an undefined origin (the local market) and hence were unlikely to be of the same age and strain. Marked differences in thyroid morphology and function between different strains of dogs and even different breeds have been reported in the literature. Capen and Martin (1989), for example, reported that – at the same level of iodine intake – thyroidal turnover of iodine in Basenji dogs (an African breed) is 2 to 3 times faster than in European canine breeds. This implies that the thyroid is more stimulated in these dogs and is likely to be more sensitive to

perturbations in iodine uptake. In addition, younger, rapidly growing dogs have a greater iodine uptake than adult dogs and may thus be even more sensitive (Capen and Martin, 1989). It is therefore possible that the observations made by the authors have been confounded by, or even reflect, inter-individual differences between the dogs rather than effects due to the different treatments (Figure 27, Section 8.0.2).

There are also concerns regarding the significance of the reported changes in T3 and whether these can be relied upon as true indication of the thyroid status of the animals. Firstly, the ratio of T4:T3 in dog plasma is normally about 20:1, so reported changes in T3 could have reflected subtle alterations in T4:T3 balance. Secondly, the time of day at which blood samples are taken is important because diurnal oscillations in plasma T3 and T4 levels have been reported in dogs (Capen and Martin, 1989). Thirdly, the reported elevated T3 levels in the cassava group pre-dosing are suggestive that there were study variables that were not being adequately controlled.

Furthermore, there were some inconsistencies in the statistical analysis of data presented by Kamalu and Agharanya (1991). Therefore, the Task Force made a renewed analysis of these data. In Figure 1 of Kamalu and Agharanya (1991), only 3 data were presented for 6 dogs of both treatment groups, so it was assumed that there was overlap of the same data points for 2 dogs. These were treated as separate data. Regression analysis (using Excel software) provided the following results (ten Berge, 2003b):

- The intercept (average T3 serum level of the control group) was found to be 1.25 and the standard deviation 0.15. This standard deviation is clearly different from the standard deviation presented in Table 1 of Kamalu and Agharanya (1991) for the control group. The plotted T3 values of the control group show such a wide variation that the variation is not consistent with a standard deviation of 0.05.
- Further the coefficient of regression of the thiocyanate plasma level on the total serum T3 level appeared to be not significantly different from zero. A probability of 24% was found that the regression coefficient is zero or even positive.
- The T3 levels of the cassava- and cyanide-treated groups were all in the range of the control groups that varied considerably in the study.
- The study also suffers from the fact that the thiocyanate levels in the controls were set to 0 in the experiment by using them as blank controls for the determinations of thiocyanate levels in the dose groups, which are therefore also not fully reliable.
- A thorough analysis of the data on T3 levels in the study reveals that the reported findings are more likely to represent variations due to experimental deficiencies and chance variations in the tested populations rather than exposure or age-related changes in hormone levels.

The Task Force also had concerns regarding the significance of the observed pathological effects on the thyroid and the possibility that these were perhaps confounded by, or even due to, differences in dietary iodide levels. It is generally recognised that effects on the thyroid are very dependent on blood iodide levels and this in turn is dependent upon the content of iodine in the diet. The authors, mindful of this, took what they thought was reasonable precaution to control for dietary iodide by inclusion of a dietary supplement of potassium iodide. They also acknowled that the meat supplement received by the cassava group was probably an additional source of iodine (and therefore animals in this group likely had the highest level of dietary iodine). What was not perhaps adequately controlled, however, was the unexpected reduced body-weight gain reported for the dogs receiving NaCN-rice and the likelihood that they had a reduced food intake and consequently, probably the lowest level of dietary iodide of any of the groups. Considering that dogs are very sensitive to dietary iodide and that growing dogs are even more sensitive, it is not possible to exclude the possibility that the effects on the thyroid were confounded by differences in dietary iodide levels. This could perhaps explain why the histopathological changes were observed in the thyroids of the animals in the cyanide and control groups but not the cassava group (animals receiving highest iodine levels), as opposed to the cyanide and cassava groups but not the control group as might have been expected.

In conclusion, there are sufficient experimental uncertainties as to prevent any firm conclusions to be drawn from these studies.

Pig

In the context of evaluating the feeding of animals used for meat production with cassava diets, Tewe and Maner (1980) studied the effects of dietary cyanide on the growth performance of pigs and the influence of protein and iodine deficiency on pathological changes. Two groups of weanling Yorkshire pigs, one group consisting of 16 males and another of 16 males and 16 females, were randomly allocated to eight experimental treatment groups (2/sex/group) receiving diets of different composition. A diet containing sufficient protein (16%) and a protein-deficient diet (9% protein) were either supplemented with iodine salt (36 µg/100 g diet) or not supplemented. Both diets were additionally supplemented with vitamins (separately added) and other minerals. The cyanide treatment groups received 500 ppm (50 mg CN^-/100 g diet) added as KCN to the respective diet. Water and food were supplied *ad libitum*. After 14 days of treatment the male animals of both groups were moved to metabolism cages for one week were they received food *ad libitum* and water (3 ×/d). Urine was collected daily during that week and analysed for thiocyanate and iodine. The animals were then returned to their treatments and were killed at day 56 of the study. The females received the diets continuously for 56 days. Blood samples were taken from the anterior vena cava at the start of the study and every 2 weeks thereafter and were analysed for serum thiocyanate, protein bound iodine and total serum protein.

At necropsy the following organs were weighed: thyroid, spleen, liver, kidneys and heart. Samples of liver and kidney were analysed for rhodanese levels. Histopathology was performed in two animals of the second group on the following organs and tissues: thyroid, pituitary, stomach, liver, heart ventricle, anterior aorta, lympho-reticular system tissues from spleen, tonsils, thymus, intestinal mesenteries, kidneys, eye, brain, spinal cord, neural ganglion, bone tissue from 9th rib and frontal bone. The results are summarised in Table 46.

Table 46: Effects of cyanide in pigs fed protein-sufficient/deficient and (non-)iodine-supplemented diets [a] (Tewe and Maner, 1980)

	Diet (% protein, with and without iodine)				Diet (% protein, with or without iodine) and added CN^-			
Effect	**16%, + I^-**	**16%, – I^-**	**9%, + I^-**	**9%, – I^-**	**16%, + I^-**	**16%, – I^-**	**9%, + I^-**	**9%, – I^-**
Food consumption			↓	↓			↓	↓↓
Bw gain			↓	↓			↓	↓
Food efficiency (food consumption/bw gain)			↑	↑			↑	↑
Thyroid weight g/100 kgbw	8.70 ± 0.30	↑ 24.9 ± 0.13	↓ 5.85 ± 0.83	↑ 15.7 ± 8.6	12.2 ± 0.49	↑ 35.7 ± 1.70	8.83 ± 0.35	↑ 14.05 ± 1.47
Kidney weight				↓			↓	↓
Urinary SCN^- excretion (mg/kg food intake)	7.7 ± 0.1	8.6 ± 1.2	↑ 9.2 ± 1.8	8.4 ± 0.8	↑ 15.5 ± 2.2	↑ 12.8 ± 1.6	↑ 13.7 ± 4.7	↑ 12.3 ± 2.9
Urinary I^- excretion (µg/kg food intake)	88.0 ± 3.3	↓ 36.8 ± 0.7	↓↓ 5.57 ± 0.6	↓↓ 5.88 ± 0.2	↑ 104.3 ± 7.1	↓ 41.3 ± 3.9	↓↓ 6.85 ± 0.6	↓↓ 4.40 ± 0.7
Serum SCN^- level (mg/100 ml)	0.46 ± 0.02	0.46 ± 0.03	0.43 ± 0.03	0.41 ± 0.002	↑ 1.77 ± 0.19	↑ 1.81 ± 0.18	↑ 0.98 ± 0.14	↑ 1.14 ± 0.18
Serum protein (g/100 ml)	7.8 ± 0.1	7.8 ± 0.2	↓ 5.8 ± 0.1	↓ 5.6 ± 0.1	7.4 ± 0.2	8.0 ± 0.2	↓ 5.6 ± 0.2	↓ 5.8 ± 0.2
Serum protein bound iodine (µg/100 ml)	4.1 ± 0.16	↓ 3.4 ± 0.19	↓ 3.4 ± 0.13	↓↓ 2.7 ± 0.14	3.9 ± 0.22	↓ 3.5 ± 0.13	↓ 3.3 ± 0.41	↓↓ 3.0 ± 0.23

Table 46: Effects of cyanide in pigs fed protein-sufficient/deficient and (non-)iodine-supplemented diets[a] (cont'd)

	Diet (% protein, with and without iodine)				Diet (% protein, with or without iodine) and added CN^-			
Effect	**16%, + I^-**	**16%, – I^-**	**9%, + I^-**	**9%, – I^-**	**16%, + I^-**	**16%, – I^-**	**9%, + I^-**	**9%, – I^-**
Histopathological changes		Thyroid: hyperplasic goitre	Atrophy of the thyroid, enlarged cavities in bone cells (slowed bone development)	Atrophy of the thyroid (1 animal), thyroid hyperplasia 1 animal, enlarged cavities in bone cells (slowed bone development)	No pathological changes	Thyroid: hyperplasic goitre	Atrophy of the thyroid, enlarged cavities in bone cells (slowed bone development), fatty infiltrations in the liver	Atrophy of the thyroid (1 animal), thyroid hyperplasia 1 animal, enlarged cavities in bone cells (slowed bone development)

[a] ↑/↓, statistically significant ($p < 0.01$) increase/decrease compared to controls (↓↓, $p < 0.05$); no entry: no statistically significant change

Effects on body-weight gain and food consumption were mainly related to protein deficiency, and not correlated with cyanide uptake. While reduction in food consumption in the group treated with insufficient protein, iodine deficiency and cyanide was more pronounced than in the other protein-deficient groups it had no influence on the body-weight development. Iodine deficiency led to an increase in thyroid weight while protein deficiency resulted in a decrease in thyroid weight. Pigs deficient in both protein and iodine had increased thyroid weights. Addition of cyanide to the diet caused a slight (not statistically significant) increase in thyroid weights in iodine-supplemented animals that was far less pronounced than the increase induced by iodine deficiency. In iodine deficient pigs receiving additional cyanide in the diet, a further increase of thyroid weight was observed that was, however, not significantly different from the increase induced by iodine deficiency alone. Pigs receiving cyanide in their diet had elevated thiocyanate serum levels and increased thiocyanate and iodine excretion in urine. Protein deficiency reduced urinary iodine excretion, serum thiocyanate levels, serum protein and serum protein bound iodine, while iodine deficiency reduced urinary iodine excretion and serum protein bound iodine. If protein-deficient pigs received cyanide, urinary iodine excretion was reduced and kidney rhodanese activity was lowered. No changes in neuronal tissues were observed. This study showed that thyroid hyperplasia was mainly mediated by iodine deficiency and that cyanide had an additional goitrogenic effect in the animals that were fed iodine deficient diets. Cyanide increased urinary iodine excretion, but did not change the serum-bound iodine levels. Protein deficiency reduced urinary thiocyanate excretion, but also thiocyanate synthesis. Thus protein deficiency may lead to higher free cyanide levels and increased toxicity that was, however, not observed in this study.

Jackson (1988a) studied the effects of cyanide (KCN) doses that were in the order of magnitude of cyanide doses that humans would receive from cassava consumption in Liberia. Young (5-wk old) miniature pigs (3/group) received 0.4 to 1.2 mg CN^-/kgbw/d for 24 weeks. Five animals were unspayed (ovaries not removed) females; the remaining 7 were castrated males. The diets were reported to be supplemented for vitamins and minerals, but the iodine content was not indicated. The animals received the daily dose as a bolus by gavage prior to the daily meal. The controls received water in the same manner. Body weights were recorded before acclimatisation and one week later before assigning the pigs randomly to the study groups at the start of the study. The actual weights were not reported in the publication. Food and water consumption were not measured at any time. The investigations were restricted to a number of behavioural determinations performed daily and categorised as performance measures, learning events and other types of behaviour. Glucose, thyroid hormone and serum thiocyanate concentrations were determined every 6 weeks. However, it was not stated if the blood sampling was performed at the same time of the day on each occasion. In the publication, no data on thiocyanate blood and urine levels were reported. According to the author, dose-related decreases in blood T3 and T4 levels were evident in all treatment groups from week 6. There was also an increase in fasting blood-glucose, particularly in the top dose animals. Statistical analysis was not presented for each

dose group versus control, but changes in top dose animals appeared significant by week 18. By week 24, decreases of 35% for T3, 15% for T4, and an increase of 60% in fasting blood-glucose were observed. The author did, however, not give any indications of standard deviations from the means. According to the author, thiocyanate levels correlated well with the cyanide dose, but the values were not reported in the publication. Behavioural assessment revealed associations with a decrease in dominance behaviour, increases in distractibility from eating, short recovery time after anaesthesia, limping, stiffness and shivering in the high-dose group, a decrease in fighting and reduced aggression and increased vomiting in the high and mid dose groups. At all doses, increased victimisation, decreased explorative behaviour, reduced rooting and water overturning were noted. The author summarised the effects as indicating an increasing ambivalence and slower reaction response times to different stimuli with increased cyanide exposure.

The reported changes in behaviour such as exploration and aggression, slower eating, more frequent drinking and shivering may, according to the author, be consistent with a decrease in high energy-demand activities, which in turn would be consistent with the decreased thyroid activity. However, no information was provided on the free and protein-bound portion of T3 and T4. This is important because only the free portion controls the metabolism and general metabolic rate. Furthermore, these behavioural changes might also be explained in terms of a decrease in dominance behaviours consistent with general toxicity and submission to healthier, more aggressive animals, housed in the same pens. Although the LD_{50} of cyanide for this pig strain was not provided by the author, nor could it be found in the literature, it is possible to make some judgements as to the level of toxicity that might have occurred in these animals. On the basis of allometric scaling compared to the rat and assuming a body weight of 10 kg, the acute LD_{50} for these animals can be estimated to be approximately 2 mg/kgbw. On this basis the highest dose used was only a factor of 2 away from the estimated LD_{50}. Since the daily dose of KCN was given as an oral bolus that would have been rapidly absorbed from the gastro-intestinal tract it would seem reasonable to assume that some degree of repeated, daily, acute poisoning must have taken place. As the animals were housed together rather than individually, this acute poisoning may well have led to the development of social hierarchies (social disadvantage) and stress reactions in the treated pigs (compared to controls).

Significant decreases in thyroid hormones T3 and T4 were reported in the high-dose group suggestive of toxicity to the thyroid. However, there are several features that indicate that this conclusion is not reliable. Firstly, although individual animal weights were not reported, it is possible to calculate the average bodyweight of the animals at each dose level from data given on the cumulative cyanide load. Unfortunately, no data were presented for the control animals (0 mg CN^-). The calculations of the average body weights are presented below (Table 47).

Table 47: Accumulative dose and calculated mean bodyweights for KCN treated mini-pigs
(Jackson, 1988a)

Dose level:	0.4 mg CN^-/kgbw/d		0.7 mg CN^-/kgbw/d		1.2 mg CN^-/kgbw/d	
Week	Accumulative dose (mg/kgbw)	Mean bw (kg)	Accumulative dose (mg/kgbw)	Mean bw (kg)	Accumulative dose (mg/kgbw)	Mean bw (kg)
8	63	2.81	204	5.2	336	5.00
12	112	3.33	291	6.65	661	6.58
16	162	3.62	646	8.24	998	7.43
20	235	4.20	954	9.73	1,474	8.77
24	323	4.81	1,323	11.25	1,953	9.69

Although animals in all groups apparently doubled their bodyweight over the 24 weeks of the study, the mean bodyweight of the lowest treatment group (0.4 mg/kg/d) was considerably lower than that of the other two treatment groups throughout. This difference may be very significant as animals with a lower body weight have a higher metabolic demand because more energy is required per kg bodyweight to maintain an elevated body temperature. As the higher metabolic turnover in these animals is regulated by the thyroid producing more T3 and T4 it is not surprising that the lower dose group had a higher T3 and T4 than the higher dose groups. Unfortunately no bodyweight data are presented for the control animals nor was a statistical analysis presented against control animals.

Toxicokinetic analysis of the data presented by Jackson (1988a) may provide further evidence that the study is perhaps unreliable for extrapolation of the effect of cyanide to man. The dose was given (1 ×/d) in water to juvenile pigs by gavage directly before food was supplied. Modelling of the predicted absorption profile indicates that the cyanide level in blood might rise to 2.9 mg/l, a clearly toxic level that would be expected to cause dizziness, nausea and related effects, whereas the estimated serum level of thiocyanate in pigs only reached about 260 µmol/l (15 mg/l) (Appendix G). As this serum level is frequently observed in smokers (Scheuermann *et al*, 1991 cited by Knudsen *et al*, 2000, 2002; Tsuge *et al*, 2000) without obvious behavioural changes, it is unlikely that the reported behavioural effects are due to an effect of accumulated thiocyanate on the thyroid, as claimed by the author, but rather caused by repeated sublethal intoxications by unbound cyanide.

The second point of note is that, as described previously, thyroid activity is subject to influence by many factors including stress and dietary iodide. On the basis of the behavioural observations reported, and because the animals were housed and fed together, the treated animals would have

been stressed and may have received different food intakes, and therefore different levels of dietary iodide. Consequently, even if the changes in thyroid hormone level had been significant when compared with controls, the possibility could not be excluded that these differences had been confounded by, or even due to, factors associated with the study design and housing conditions rather than a true substance related effect.

Given these concerns, as well as the low animal number per group and inadequate statistical analysis, the Task Force was of the opinion that no firm conclusion can be drawn from this study with regard to a clear treatment-related effects and effect levels.

Soto-Blanco *et al* (2001a) studied possible diabetogenic effects after repeated cyanide dosing of pigs. Groups of 6 or 7 male 21-day old Landrace-Large pigs received (2 ×/d) cyanide at daily doses of 0, 2, 4 or 6 mg KCN/kgbw (corresponding to 0, 0.8, 1.6 or 2.4 mg CN^-/kgbw/d) in corn starch biscuits for 74 consecutive days. Venous blood samples were taken at the end of the experiment from the external carotid vein and were analysed for thiocyanate and glucose. Three animals of each group were necropsied at the end of the study and the pancreas was examined histopathologically. Body-weight gains of exposed pigs were not significantly different from that of controls. Thiocyanate plasma levels showed a dose-dependent increase. No effects were observed on the glucose levels and no gross or histopathological changes were observed in the pancreas.

Goat

The same authors performed a similar experiment in goats. Alpine-Saanen goats (6 - 8 castrated males/group, aged 30 to 45 days at the beginning of the experiment) received (2 ×/d) 600 ml of milk, 100 g concentrate (not specified) and Napier grass (*Pennisetum purpureum*) *ad libitum* for the first 3 months. Cyanide was administered (2 ×/d) at 0, 0.3, 0.6, 1.2 or 3 mg KCN/kgbw/d (0, 0.12, 0.24 0.48 or 1.2 mg CN^-/kgbw/d) for 5 months, in milk during the first 3 months until weaning, thereafter by gavage in tap water. The iodine content of food or urine was not stated. Body-weight gains were lower compared to controls in the 3 mg/kgbw dose group. Thiocyanate plasma levels were elevated at 3 mg/kgbw after 3 and 5 months. In the controls, there was a decrease of SCN^- plasma levels during the study. No effects were observed on the glucose levels and no gross or histopathological changes were observed in the pancreas. Plasma samples at the end of the study were analysed additionally for T4 and T3 by radioimmunoassay. Blood parameters such as erythrocyte counts, leukocyte counts, packed cellular volume and haemoglobin were also determined. The authors reported generalised tremors and ataxia in one animal 30 seconds after dosing in the period of day 121 to day 123 (gavage phase). This indicated high acutely toxic dose levels after the gavage exposure. No differences were found in the plasma T4 levels at any dose. The plasma T3 levels were significantly different from controls in the

high-dose group only. The thyroids of the high-dose group showed an increased amount of re-absorption vacuoles in the follicular colloid. Some re-absorption vacuoles were also found in the 1.2 mg/kgbw dose group. The plasma thiocyanate levels in the 1.2 mg/kgbw dose group were however not elevated during the course of the study. The number of erythrocytes, haemoglobin concentrations and packed cell volume were decreased at 1.2 mg/kgbw compared to controls (Soto-Blanco *et al*, 2001a,b).

8.3.2 Dermal

No data are available.

8.3.3 Inhalation

Details and results of the available repeated inhalation studies are presented in Table 48.

Table 48: Repeated inhalation toxicity

Species / Strain, number and sex/group	Test substance	Concentration [a] (ppm)	Concentration [a] (mg/m^3)	Exposure regime	Dose (mg CN^-/kgbw/d)	Duration	Result	Remark	Reference	CoR
Rat										
Sprague-Dawley, 10 M, 10 F	ACH	0 9.2 29.9 59.9	(0 32.5 106 212)	6 h/d, 5 d/wk	0 1.3 4.4 8.7 [b]	4 wk	M at 59.9 ppm: mortality 3/10. Prior to death: respiratory distress, tremors, and convulsions. M + F at 59.9 and 29.9 ppm: irritation of eyes and nose, breathing difficulties. All exposures: ↑ serum and urine thiocyanate levels. Changes in clinical chemistry and haematology parameters within range of historical controls. No gross or microscopic lesions attributable to ACH exposure. NOAEL = 9.2 ppm		Monsanto, 1981d	1b
Sprague-Dawley, 15 M, 15 F	ACH	0, 10.5 32.3 61.2	(0 37.1 114 217)	6 h/d, 5 d/wk	0 1.8 5.5 10.4 [c]	14 wk	No exposure-related signs of toxicity, no changes in haematological parameters. 10.5, 32.3, ppm: ↑ serum thiocyanate blood levels. All exposure: ↑ urine thiocyanate levels. No exposure-related gross or microscopic changes. NOAEL = 61.2 ppm		Monsanto, 1984	1b

Table 48: Repeated inhalation toxicity (cont'd)

Species / Strain, number and sex/group	Test substance	Concentration [a] (ppm)	Concentration [a] (mg/m^3)	Exposure regime	Dose (mg CN^-/kgbw/d)	Duration	Result	Remark	Reference	CoR
Rabbit										
Danish county breed, albino, 8 - 22 M	HCN	0 0.5	(0 0.56)	Continuous	-	4 wk	No microscopically detectable changes in myocardial ultrastructure	Whole-body exposure. Measured atmospheric concentration 2 ×/d. Investigation restricted to myocardial changes	Hugod, 1979	3a

Table 48: Repeated inhalation toxicity (cont'd)

Species / Strain, number and sex/group	Test substance	Concentration [a] (ppm)	Concentration [a] (mg/m^3)	Exposure regime	Dose (mg CN^-/kgbw/d)	Duration	Result	Remark	Reference	CoR
Dog										
Dog (strain not stated), 4 (sex not stated)	HCN	(44.5)	50	12 × 30 min/ 8 d 14 × 30 min/ 2 d 19 × 30 min/ 2 d	-	96 d 28 d 38 d	5/17 deaths. Dyspnoea, vomiting, diarrhoea, muscular tremors, affected locomotion, affected equilibrium, general convulsions and tonic contraction of limbs. Brain pathology: vascular lesions, cellular lesions in cerebellum and medulla oblongata, glial reaction. Purkinje cell lesions in cerebellum	Repeated acute exposure to near lethal levels. No controls. Limited reporting	Valade, 1952	3c

a Converted values in parentheses

b 0, 9.9, 32.3, 64.8 mg CN^-/m3. Dose calculated using minute volume of 150 ml/min and average bw of 200 g/rat (ECB, 2003 update defaults), assuming 50% absorption

c 0, 11.3, 34.9, 66.3 mg CN^-/m3. Dose calculated using minute volume of 175 ml/min and an average bw of 275 g/rat (ECB, 2003 update defaults), assuming 50% absorption

Subacute

Sprague-Dawley rats were exposed (whole-body) to ACH vapours at concentrations of up to 59.9 ppm (measured) for 4 weeks (28 days). The test atmospheres were generated by bubbling nitrogen gas through liquid ACH into an airflow (1,643 l/min) for low exposure (9.2 ppm) or by nebulising pressurised ACH for medium (29.9 ppm) and high (59.9 ppm) exposures, both into an airflow of 1,727 l/min. The air concentrations were monitored by infra-red spectrophotometry of samples taken (4 ×/d) at a port in the centre of the exposure chamber door.

Clinical observations were made daily prior to, between 2 and 5 hours during, and after exposure. Observations during exposure included irritation of the eyes and/or nose and breathing difficulties in all animals at 29.9 and 59.9 ppm. In rats at 59.9 ppm, hypoactivity was observed. Soon after commencement of exposure, 4 males at 59.9 ppm showed symptoms of anoxia such as respiratory distress, tremors or convulsions, foam around the mouth and prostrate posture. Three of the 10 high-dose males subsequently died. An additional observation (pre- and post exposure and weekly) was chromo-rhinorrhoea that appeared more frequently in rats at 59.9 ppm and less at 29.9 ppm, but also on single occasions in controls and at 9.2 ppm. The mean body weight (recorded weekly) of the males at 59.9 ppm was lower than that of controls, but the difference was not statistically significant. The body-weight development of the other exposure groups did not differ from that of the controls.

At necropsy, haematological examination revealed statistically significant decreases in red blood cell counts, haemoglobin and mean corpuscular haemoglobin concentrations in females at 59.9 ppm compared to controls, but the levels were still in the range of historical control data. Females at 59.9 ppm also revealed a significant increase in blood urea nitrogen (BUN) and a decrease in lactate dehydrogenase (LDH1). Total serum protein levels were statistically significantly decreased in males at 29.9 and 59.9 ppm. In the males at 29.9 ppm, a significant decrease in lactate dehydrogenase levels was also noted. All these changes were within the biological variation of this rat strain and were not considered treatment-related by the authors. Serum thyroid hormone (T3 and T4) levels were not significantly different from controls except for the 29.9 ppm males that showed elevated T3 levels. Increases in serum thiocyanate and urinary thiocyanate excretion were observed in all test groups. The increases in serum thiocyanate reached statistical significance in rats of both sexes at 9.2 and 29.9 ppm and the males at 29.9 ppm, while the increase in urinary thiocyanate excretion was significant in animals at 29.9 and 59.9 ppm (Appendix D). The terminal body weights were reduced in males at 59.9 ppm only, but the reduction was not statistically significant. No changes in absolute organ weights were observed, but there were increased relative liver weights in males at 29.9 and 59.9 ppm (statistically significant only at 29.9 ppm), but these changes were within normal biological variation and a result of the decreased terminal body weights. There were no exposure related gross or microscopic pathological changes of the following organs: adrenals, bone and bone

marrow (femur), optic nerves, testes, heart, intestines and duodenum, kidney, liver, lung, nasal turbinates, spleen, skeletal muscle, thyroid, trachea at 59.9 ppm compared to controls. The NOAEL of the study was reported to be 9.2 ppm based on the clinical observations. The LOEL was 29.9 ppm (Monsanto, 1981d).

The LOEL is based on the clinical observation of local irritation of the eyes, nose and respiratory tract. Test substance related systemic effects were not observed at 29.9 ppm. Thus the NOAEL for systemic effects would be 29.9 ppm in this study. During the first day of exposures the chamber concentrations were reported to fluctuate considerably. At the four measurement times during day one a level of 60 ppm was exceeded 3 times with a maximum concentration of 63.5 ppm. In all other measurements of the high-dose group at the following days the levels stayed mostly below 60 ppm and only occasionally went to a maximum 61.5 ppm. Deaths that occurred following exposure on day 1 were obviously due to acute toxicity and reflect the steep dose response for cyanide and ACH and the difficulty in designing repeated dose studies without exposing the animals to acutely lethal doses. A meaningful systemic NOAEL for repeated dose toxicity can therefore not be derived from this study.

Subchronic

Sprague-Dawley rats were exposed to ACH vapours at concentrations of up to 61.2 ppm (measured) for 14 weeks (90 days). Pressurised ACH was nebulised into an airflow (1,699 l/min) to generate low (10.5 ppm), medium (32.3 ppm) and high (61.2 ppm) exposures. The air concentrations were monitored by infra-red spectrophotometry of samples taken (4 ×/d) at a port in the centre of the exposure chamber door. Even distribution of the test material in the chamber was verified at regular intervals. No deaths occurred during the course of the study. Clinical observations were made daily prior to, between 2 and 5 hours during, and after exposure. Nasal discharge, salivation, integument and swaying movements were noted before and after exposure. These effects were not considered to be treatment-related by the authors since they occurred with a similar incidence in the control group or only at single times in individual animals, and were not related to concentration. No statistically significant decrease in mean body weight (recorded weekly) was observed in the exposed animals compared to controls.

Following necropsy, the slight changes seen in haematological parameters, in particular red blood cell count, were not statistically significant and within the background variation of the Sprague-Dawley rat strain used. In females exposed to 32.3 and 61.2 ppm ACH, a statistically significant decrease in blood glucose levels was observed. In these females, there were also slight (statistically significant) decreases in globulin levels compared to controls. In females at 10.5 ppm and 32.3 ppm, total serum protein levels were decreased. The observed changes in the clinical chemical parameters were within the historical background ranges and not considered

treatment-related by the authors. Increases in urinary thiocyanate levels compared to control animals were observed in a dose-related manner, but these only reached statistical significance in rats at 32.3 and 61.2 ppm. A statistically significant elevation of serum thiocyanate levels was only observed in female rats at 10.5 and 32.3 ppm (Appendix D). The serum levels fluctuated considerably and were not clearly dose related. (This gives rise to some doubt about the appropriateness of the analytical method used for the determination of the serum SCN^- concentrations.) No changes in serum T3 or T4 levels or in serum protein fractions were observed in the treated animals compared to controls. There were no treatment-related changes in body weight or organ weights. Neither were there macroscopic or microscopic changes of the following organs in rats at 61.2 ppm compared to controls: adrenals, bone and bone marrow (femur), optic nerves, testes, heart, intestines and duodenum, kidney, liver, lung, nasal turbinates, spleen, skeletal muscle, thyroid and trachea. The following additional organs were also unchanged: oesophagus, eyes, ovaries, epididymis, mesenteric lymph nodes, mammary glands, pancreas, pituitary, prostate, salivary gland, sciatic nerves, skin, spinal cord, stomach, thymus, trachea, parathyroid and urinary bladder. The NOAEL was 61.2 ppm ACH (216 mg/m^3 or 66 mg CN^-/m^3), the highest concentration tested (Monsanto, 1984). This corresponds to approximately 10.4 mg CN^-/kgbw/d.

Contrary to the 28-day study (Monsanto, 1981d), this 90-day study did not reveal any significant local irritating effect of the test substance when compared to controls. This could be due to the fact that a different batch with perhaps another spectrum of impurities (e.g. acid stabiliser content) was used, but no firm conclusions can be drawn from the report. With regard to systemic toxicity the fluctuations of the test substance concentrations were better controlled in the 90-day study compared to the 28-day study and did not lead to inhalation of acutely lethal doses by the test animals. To elucidate this further, the Task Force calculated the lethal doses in the 28-day study for the animals that died following day one and for animals exposed to the highest single concentration in the 90-day study (on day 52) (Appendix K).

8.3.4 Other routes

Subcutaneous

CF-1 mice received (1 ×/3 d) by *s.c.* injection 4 mg/kgbw of KCN for up to 30 days. One group additionally received (1 ×/d) S-adenosyl-L-methionine (15 mg/kgbw *s.c.*) for 30 days. Vehicle control groups (saline, *p*-toluene sulphonate) were included in the study. Animals were killed at different time intervals (4, 10, 16, 23 and 30 days). Liver, brain and heart were removed homogenised and processed by differential centrifugation. Rhodanese and δ-aminolaevulinic acid dehydratase (ALAD) activities were determined in the 15,000 × g supernatant. Tissue cyanide and polythionate ('cyanide-labile sulphur') concentrations were measured as well. Liver, brain

and heart cyanide levels remained unchanged with time. Blood cyanide concentrations increased with time. No difference in cyanide blood or tissue concentrations was observed between rats treated with KCN alone and those receiving S-adenosyl-L-methionine additionally. Liver and brain rhodanese activity was reduced (60% and 55%, respectively) with time in animals receiving cyanide, cyanide plus S-adenosyl-L-methionine and in the S-adenosyl-L-methionine controls. There was no significant change in rhodanese activity in the heart. Shortly after onset of treatment, cyanide-labile sulphur levels were increased in liver (cyanide, S-adenosyl-L-methionine groups) and decreased thereafter. In the cyanide plus S-adenosyl-L-methionine group, the levels remained higher up to day 23 and returned to normal by day 30. In all three groups, the brain levels of cyanide-labile sulphur increased by 80% over the whole study period. In the heart, the same level was reached after 10 days; it then returned to normal. An increase of 58% of cyanide-labile sulphur was seen in the blood over the whole study period. ALAD activity was reduced in liver and blood of cyanide-treated animals by 55% or 42%, respectively. With additional S-adenosyl-L-methionine treatment, the reduction of ALAD activity was only 35%. With S-adenosyl-L-methionine alone, no change in ALAD activity was observed. The authors concluded that one of the earliest effects of cyanide intoxication was the inhibition of rhodanese activity in liver, but not in blood (Buzaleh *et al*, 1990a,b; CoR 2e).

Crampton *et al* (1979; CoR 2e) studied the effect of repeated *s.c.* administration of KCN in baboons (*Papio cynocephalus* or *Papio anubis*) for 42 months. The baboons (about 9 months old, 7 - 10 males/group) were fed a cobalamin deficient diet. The animals received (1 ×/wk) an intramuscular injection of cobalamin (10 µg) or cobalamin (1 ×/wk) plus a daily dose of 1 mg KCN/kgbw (5 d/wk) *s.c.*, or were injected (5 d/wk) *s.c.* with cyanide (1 mg KCN/kgbw/d) or potassium thiocyanate (40 mg KSCN/kgbw/d). The latter two groups remained without additional cobalamin. Controls were without cobalamin, cyanide or cyanate. The study parameters were restricted to haematology, electroencephalography, electromyography and peripheral nerve conduction time. At necropsy, histopathological examinations included light microscopy of the cerebral hemispheres, brain stem, cerebellum, spinal cord and posterior root ganglia, and electron microscopy of the median nerves. No differences between the groups in clinical signs and body-weight development were observed. Haematology revealed an increase in haemoglobin levels in controls, cobalamin, and cobalamin plus cyanide-treated baboons, and a decrease in packed cell volume in the cyanide and thiocyanate treated groups without cobalamin supplement. The cyanide-treated baboons without cobalamin had a reduced white blood cell count. In cobalamin-supplemented baboons, plasma cobalamin was increased in the cyanide-receiving group. Plasma thiocyanate levels were highest in the cyanide-treated group without additional cobalamin followed by the thiocyanate group (that received on a molar basis a 27 times greater dose than the cyanide-treated animals) and the cyanide plus cobalamin group. No neurological changes were observed in any of the treatment groups.

Smith *et al* (1963; CoR 3c) administered (1 ×/wk) cyanide (0.5 mg/animal) by *s.c.* injection of two groups of Wistar rats (3 adults each) for 22 weeks; 3 rats served as controls. No clinical symptoms were observed during treatment. Upon termination, blood and liver were examined for vitamin B_{12} and foliate activity, and the brain was evaluated histopathologically. Cyanide-treated animals showed neuronal degeneration of the hippocampus and cerebellum. Liver vitamin B_{12} levels in treated animals were lower than in controls, but the authors considered the study too limited to draw any firm conclusions on a vitamin B_{12} depleting effect of cyanides.

Intraperitoneal

Effects on memory of chronic sublethal cyanide treatment were studied in Wistar rats (10 males/group). The rats were injected *i.p.* with NaCN solution at daily doses of 2 mg/kgbw (resembling 1/4 of the LD_{50}) for 30 days. Controls were injected *i.p.* with the same volume of normal saline. The rats received normal or protein-reduced diets. Included were malnourished rats receiving 75% potato and 25% normal diet. Memory was tested weekly using the T-maze test. Each animal had 10 trials per test and the percentage of correct trials was recorded. Precautions were taken to exclude interference by the operator or other external stimuli. (It is not clear from the publication if the performance in the test was also measured before NaCN administration.) At the end of the study, the animals were killed by cervical dislocation. Neurotransmitters that were considered important for memory functions (dopamine, noradrenaline, adrenaline and 5-hydroxytryptamine) were analysed in the dissected hippocampus area of the brain by HPLC.

No deaths, effects on body weight or clinical symptoms were observed in rats treated with cyanide compared to untreated controls. The malnourished, untreated group did not show significant decreases in body weight or serum protein levels compared to the normal diet control group. The authors claimed that chronic cyanide administration reduced both acquisition (first performance in a T-maze) and memory significantly, and that the effect was more pronounced in malnourished rats. The effect seemed to be increased with time in malnourished rats receiving cyanide. No difference was seen between normally fed rats and malnourished controls. (The Task Force has re-evaluated the results, tabulated in the paper, of the behavioural test after 30 minutes and 30 days of cyanide treatment. The conclusion was that, in rats receiving the normal diet, there was no significant difference in performance between acute and repeated exposure.)

In the malnourished controls, noradrenaline (norepinephrine) levels were significantly increased and serotonin (5-hydroxytryptamine) levels were significantly decreased compared to normal diet controls. Cyanide treatment led to significantly lower levels of dopamine and serotonin, and higher levels of noradrenaline and adrenaline, when compared to controls. The effects were increased in the malnourished animals. According to the authors, this was consistent with other

studies indicating a reduction of dopamine and serotonin levels in the hippocampus, which was associated with deficits in memory and operant conditioning performance (Mathangi and Namasivayam, 2000). From the study it is unclear if the described effects are of an acute or chronic nature.

8.3.5 Summary

Repeated dose toxicity studies on cyanides of relevance for the human exposure situation have been conducted by the oral and inhalation route. Some of the oral studies were designed to evaluate the impact of cassava diets on animal health and included groups that received cyanide supplemented food diets for comparison of the effects.

As cyanide is rapidly absorbed via all routes it would be reasonable to expect that, other than for local effects, the findings of repeated-dose studies would be independent of route of exposure. Interestingly, the available studies appear to fall into two categories.

The first category consists of the three 90-day guideline studies in rats and mice in drinking water (Hébert, 1993) and in rats by inhalation (Monsanto, 1984), and the 1-year study in rats in diet (Philbrick *et al*, 1979). The available NOAELs from these studies are broadly consistent: 25.6 mg CN^-/kgbw/d in mice (Hébert, 1993), 12.5 mg CN^-/kgbw/d in rats (excluding reproductive effects, Section 8.6) (Hébert, 1993) or approximately 10.4 mg CN^-/kgbw/d (Monsanto, 1984) in rats. These doses are appromating the point on the steep dose-response curve at which acute lethality might be expected as suggested by the signs of acute toxicity and mortality seen in the Monsanto (1981d) 28-day study at 59.9 ppm (212 mg/m^3 or 65 mg CN^-/m^3, equivalent to 8.5 mg CN^-/kgbw/d).

The second category consists of the 24-week dietary study in miniature pigs (Jackson, 1988a) and the 14 week dietary study in dogs (Kamalu, 1991, 1993; Kamalu and Agharanya, 1991). These studies reported effects on the thyroid and thyroid hormones at doses that are approximately one order of magnitude lower than those that were apparent NOAELs in the studies in the first category (i.e. Jackson reported decreased T3 and T4 blood levels in groups of 3 pigs receiving 0.4 or 1.2 mg CN^-/kgbw/d and Kamalu and Agharanya reported goitrogenic effects on the thyroid and reduced T3 levels in dogs receiving ≈ 1.76 mg/kgbw/d). These studies have a number of deficiencies, in particular with regard to the control of the dietary intake of iodine and protein and some of the observed effects may be more related to those parameters than to cyanide toxicity alone.

From the studies of Leuschner *et al* (1991) in rats and Tewe and Maner (1980) in pigs it can be deduced that serum thiocyanate levels of about 20 µg/ml consistently did not have goitrogenic

effects on the thyroid if the diet was not iodine deficient. In the Monsanto inhalation study, levels up to about 80 µg/ml serum did not induce any effects on the thyroid or thyroid hormone levels. In the case of iodine deficiency the same doses can, however aggravate the goitrogenic effect as shown by Tewe and Maner (1980).

8.3.6 Discussion and evaluation

As noted above, the repeated-dose studies apparently fall into two distinct categories: those that relate thyroid effects to cyanide exposure and others that do not observe this effect up to generally toxic dose levels. An investigation into the possible reasons for this is required before a conclusion can be drawn with any confidence about the likely critical health effect (and NOAEL) in humans chronically exposed to cyanides.

The apparent NOELs for general toxicity related to the mechanism of inhibiting oxygen utilisation in particular in organs with high oxygen demand, such as brain and testes in the first group of studies is higher than (approximately double) the acute LD/LC_{50} values reported in rats using the same routes of exposure (Section 8.1). This can be explained by the kinetics of the detoxification reactions. It is understood that sustained, lower-level exposures to CN are better accommodated by the detoxification mechanisms (conversion to thiocyanate) in animals. Acute exposure to levels that are above the detoxification capacity will result in direct HCN-mediated cell toxicity and death particularly in tissues with a high oxygen demand, such as the brain. Hence, by spreading the applied dose over a longer exposure period a higher total dose per day can be tolerated. Interestingly, this is the reason why deaths occurred in the subacute inhalation study with ACH conducted by Monsanto. In these studies minor fluctuations in the delivered ACH concentration resulted in acute lethality in some animals. In the first group of studies no effects on the thyroid were observed, although this organ was carefully examined. Thus the apparent grouping is unlikely to be simply due to different investigators measuring different end-points. Philbrick *et al* (1979) reported significant decreases in T4 levels in rats receiving 40 or 54 mg/kgbw of cyanide in the diet after 4 months, but not after 11 months. They also noted significant increases in thyroid weights, but no histopathological alterations, after 11 months of treatment. The thyroid was also assessed in the guideline studies of Hébert (1993) and Monsanto (1984), and showed no effects at levels an order of magnitude higher.

Thyroid hormone secretion is highly regulated and maintained at different base levels and species differences in this regulation have been reported. There is some evidence that suggests this grouping is unlikely to be solely due to differences in species sensitivity between rodents and miniature pigs and dogs.

Both rats and humans have a non-specific low affinity carrier of thyroid hormone, but humans and other primates, as well as dogs, also have T4 binding globulin, a protein that binds predominantly T4 with high affinity. Rats lack T4 binding globulin and as a consequence the biological half-life of thyroid hormones in rats is about 10 fold shorter than in humans. Consequently, any interference with T4 metabolism, even a transient one, leads to a more rapid depletion of the hormone in the rat, followed by TSH secretion by the pituitary gland to stimulate the thyroid to synthesise more T3 and T4 (Zbinden, 1987; Hard, 1998; Hill *et al*, 1998). This would suggest that the rat is more sensitive than humans to changes in thyroid hormone levels. Indeed, rodents have been reported to be particularly sensitive to thyroid enlargement (Zbinden, 1987; Hard, 1998; Hill *et al*, 1998).

In dogs, iodine and thyroid hormone turnover is higher than in humans and the binding to T4 binding globulin is weaker leading to a higher globulin-bound fraction in humans. This would suggest that the dog is also more sensitive than humans to changes in thyroid hormone levels. However, no effects on the thyroid were observed in dogs exposed repeatedly for 14.5 months to acutely toxic cyanide concentrations (Hertting *et al*, 1960). This apparent inconsistency could be explained by the fact that considerable differences in hormone turnover related to strain and age have been reported in dogs (Capen and Martin, 1989). There may however be other equally plausible explanations. For example, the fact that Kamalu and Agharanya only studied T3 levels and observed changes in these levels may not have given a true reflection of overall thyroid activity. This would be because most of the thyroid hormone in the serum of all species is T4 which is converted to the more active T3 at the target organs. Normal T3 levels have much wider variations, including circadian fluctuations, and are also sensitive to stress and starvation that may transiently inhibit mono-deioinisation of T4 to T3 (Larsen, 1982; Van Leeuwen *et al*, 1987). Thus a reliable determination of the thyroid hormone status could only be based on determinations of both T3 and T4. As the dogs used by Kamalu and Agharanya were collected from the local market (therefore unlikely to be controlled for age or strain), displayed reduced weight-gain (possibly indicating starvation) and showed behavioural changes (consistent with stress) it is not possible to make firm conclusions regarding the significance of the changes in T3 hormone levels or to make generalisations about the relative sensitivity of the thyroids of the dogs used in this study.

Although no data could be found regarding the relative sensitivity of the thyroid in miniature pigs, the Task Force considered that it was likely to be comparable to that of humans.

The overall picture on inter-species sensitivity with regard to effects on the thyroid is therefore complex. The literature is, however, not consistent with the rat being apparently less sensitive than dogs and miniature pigs. On the contrary, it is well established (as outlined above) that the rat is generally regarded as more sensitive to thyroid effects than other species.

One feature that does distinguish the 'dietary' study of Jackson (1988a) from that of Kamalu and Agharanya (Kamalu, 1991, 1993; Kamalu and Agharanya, 1991; Ibebunjo *et al*, 1992) is that administration was by bolus gavage. This may be important in understanding why these studies identified effects on the thyroid at such low levels. The literature on dietary effects of cyanides (cassava) in humans (Section 9.2.2) clearly identifies the importance of dietary iodide in protecting the thyroid against cyanide-mediated toxicity. Although both investigators apparently had taken measures to address this issue, the Task Force believed that there were two key technical considerations that may have compromised the study designs. Firstly, there were incorrect assumptions regarding the profile of dose delivery. Secondly, the design and conduct of repeated-dose studies with cyanides are always extremely challenging due to the need to balance the objective of demonstrating significant chronic toxicity with the steep dose-response curve for acute toxicity.

The first point is that, in both studies, cyanide was administered in the form of a salt, either as a single oral bolus or as a food supplement. In both cases, the cyanide was in free form and would have been rapidly absorbed from within the gastro-intestinal tract. The profile of absorbed cyanide would peak shortly after dosing and contrasts with the slower, sustained release and absorption that occurred after dietary ingestion of cassava. The significance of this is that the peak in absorbed cyanide exceeded the limited detoxification (conversion to thiocyanate by rhodanese) capacity of the body, resulting in daily acute poisoning. These effects were likely to be due to inhibition of intracellular oxygen utilisation and cell death, particularly in organs with a high oxygen demand, such as the brain and testes (Section 8.0.1). The dose response for the acute effects would have been effectively superimposed over the dose response for those effects that should be associated with circulating thiocyanate, the physiological mechanism for detoxification during sustained lower-level chronic exposure, thereby masking them.

The second consideration relates to further confounding in that repeated acute poisoning might have affected normal behaviour, caused stress and led to reduced food intake/weight-gain. This could explain why effects on the thyroid were not observed in other repeated-dose studies unless dietary intake and weight gain were markedly affected, such as in the case of the higher dose groups in the study of Philbrick *et al* (1979). As described previously, the regulation of blood thiocyanate levels and their effect on the thyroid is complex. Thiocyanate is known to induce goitre in animals and humans if the blood iodine concentration is low. It can competitively inhibit iodine uptake into the thyroid gland (Turner and Bagnara, 1971). The latter can, however, be counteracted by adding iodine to the diet. Plasma thiocyanate levels will depend on the balance between its formation, by the rhodanese reaction, and its excretion, predominantly via the urine. Thiocyanate formation is also dependent on the protein status and will be lower in protein (sulphur amino acids) deficient situations. Renal clearance can vary widely and is adapted when the serum levels increase (Wood, 1975). Thus the effect of thiocyanate on the thyroid is dependent on a number of factors that can be affected during acute poisoning.

In both studies, some of the effects reported were consistent with repeated acute poisoning and this could have resulted in reduced iodine intake, an imbalance in thiocyanate formation/clearance and an effect on the thyroid. If this were the case then it is debatable whether the effects on the thyroid are truly reflective of chronic low-level exposure or, because of inadequacies in experimental design, are due to a combination of a shift in the dose response, dietary deficiency and stress, for example.

Given the above-mentioned concerns, effects reported in animal studies on thyroid hormone levels or thyroid enlargement should be treated with caution. Only if dietary iodine and protein levels, age, strain, food consumption and housing conditions are carefully controlled and comparable between the study groups, can reliable conclusions be drawn on treatment-related effects.

When reviewing the existing studies on cyanides, the Task Force concluded that the only well controlled repeated-dose studies are the 90-day drinking-water study in rats and mice by Hébert (1993) and the 90-day inhalation study in rats by Monsanto (1984). Although it has been argued that the rat may not be an ideal surrogate for humans for some end-points, it appears to be more sensitive with regard to goitrogenic effects and therefore has an in-built margin of safety with regard to likely effects in humans. In those three studies, no effects on the thyroid weight or histopathology have been observed up to the highest dose tested. In the Monsanto study no effects were observed on thyroid hormones up to the highest dose tested and the NOAEL for thyroid effects seems to be higher than that for general toxicity. Furthermore, as the administered dose levels were limited by the acute toxicity of cyanide it can be concluded that thyroid toxicity will not occur in animals at concentrations that are not acutely toxic.

The papers of Jackson (1988a), and Kamalu and Aghyranya (cited above) suggest that in the case of nutritional imbalances, and in particular iodine deficiency, effects on the thyroid may be expected at lower dose levels. However, the Task Force believed that the currently available data in animals is too weak to enable the determination of a threshold for this concern.

8.4 Genotoxicity

For HCN, its Na- and K-salts and ACH, a variety of genotoxicity indicator tests have been performed. Some of these tests followed unusual protocols while others were guideline studies. Between these studies, all conventional endpoints have been covered.

8.4.1 Genotoxicity indicator tests

Several tests on the inhibition of DNA synthesis (replicative synthesis and repair) have indicated that DNA replication in mammalian cell cultures and bacteria, is arrested at concentrations between 1 and 10 mmol/l (26 - 260 µg CN^-/ml) (Cleaver and Painter, 1975; Budzicka *et al*, 1981; Weigel and Englund, 1975) (Table 49). This has been shown to be caused by depletion of the ATP pool after inhibition of cytochrome oxidase (Weigel and Englund, 1975). Weigel and Englund also showed that anaerobic cultures are less sensitive and that further enzyme systems – including DNA replication – are also inhibited, albeit at higher cyanide concentrations.

In a number of tests, genotoxic effects due to cyanide have been found, including induction of bacterial DNA repair (De Flora *et al*, 1984a,b); DNA fragmentation, measured by ultracentrifugation (Bhattacharya and Rao, 1997) and induction of DNA double strand breaks (Vock *et al*, 1998), in the presence or absence of metabolic activation (Table 49).

Table 49: Genotoxicity indicator tests

Species, strain	Protocol	Metabolic activation	Concentration (µg KCN/ml)	Concentration (µg CN^-/ml)	Result [a]	Remark	Reference	CoR
Bacteria								
E. coli, WP67, CM871, WP2	Microwell DNA repair test with Trp-auxotrophic strains	Aroclor-induced rat liver S9 [b]	195 - 1,560 (– S9), 3,130 (+ S9)	78 - 623 (–S9), 1,250 (+ S9)	**+ve**	Induction of DNA repair via SOS pathways. Maximum inhibition at 39 - 312 µg (– S9) or 625 µg KCN/well (+ S9) [16 - 125 (– S9) or 250 µg CN^-/well (+S9)]	De Flora *et al*, 1984a,b	2e
E. coli, 15TAM	DNA synthesis inhibition test with a strain auxotrophic for Thy/Arg/Met	None	130 - 977 [c]	52 - 390	Only toxicity	Almost immediate arrest of DNA synthesis at 52 µg CN^-/ml; anaerobic cultures affected at 130 µg CN^-/ml (partial inhibition). At 390 µg CN^-/ml complete arrest	Weigel and Englund, 1975	2e
Genotoxicity tests - mammalian cells								
HeLa cell line	DNA synthesis inhibition	Aroclor-induced rat liver S9	Not stated (KCN)	Not stated	**–ve**	Reversible inhibition of DNA synthesis, interpreted as a non-genotoxic effect	Painter and Howard, 1982	2e
HeLa cell line, peripheral human lymphocytes	DNA synthesis inhibition, repair synthesis inhibition	Aroclor-induced rat liver S9	5, 50, 500, 5,000 [d]	2 - 2,000	**–ve**	Similar inhibition of repair and replicative DNA synthesis, interpreted as a non-genotoxic effect	Cleaver and Painter, 1975	2e

Table 49: Genotoxicity indicator tests (cont'd)

Species, strain	Protocol	Metabolic activation	Concentration (µg KCN/ml)	Concentration (µg CN^-/ml)	Result [a]	Remark	Reference	CoR
Mouse lymphoma L5178Y cell line	DNA synthesis inhibition	None	65 [b]	26	**–ve**	Inhibition of DNA synthesis; inhibition of oxygen uptake	Budzicka *et al*, 1981	2e
Rat, primary hepatocytes	Quantification of DNA strand breaks by alkaline elution and pulse field gel electrophoresis	Implicit	20 - 650 [c]	8 - 260	**–ve**	Cytotoxicity, but no genotoxicity were observed	Storer *et al*, 1996	2e
Rat, primary thymocytes and BHK-21, a hamster kidney cell line	Quantification of DNA strand breaks by cell lysis, ultracentrifugation and fluorometric analysis	None	81 - 650 [c]	32 - 260	**+ve**	Cytotoxicity and DNA fragmentation were observed: a concentration-dependent increase of DNA fragments in the supernatant between 1.25 and 15 mmol/l (33 - 260 µg/ml CN^-)	Bhattacharya and Rao, 1997	2e
Human A549 lung carcinoma cell line	Quantification of DNA double strand breaks by pulse field gel electro-phoresis	None	20 - 650 [c]	8 - 260	**(+ve)**	Cytotoxicity assessed by MTT [e], DNA double strand breaks occurred only in association with overt cytotoxicity	Vock *et al*, 1998	2e
			(µg NaCN/ml)					
Mouse lymphoma L5178Y cell line	Quantification of DNA single strand breaks by alkaline unwinding	None	2.5 - 98 [c]	1.9 - 39	**–ve**	No effect in the range of concentrations tested	Garberg *et al*, 1988	2e

Table 49: Genotoxicity/indicator tests (cont'd)

Species, strain	Protocol	Metabolic activation	Concentration (mg KCN/kgbw)	Concentration (mg CN^-/kgbw)	Result [a]	Comment	Reference	CoR
Rodents - genotoxicity *in vivo*								
Mouse, Swiss, 8 animals	Inhibition of testicular DNA synthesis, measured by [^{3}H]thymidine uptake	Implicit	2.5	1	**–ve**	[^{3}H]thymidine uptake comparable to control	Friedman and Staub, 1976	2e

[a] +ve, positive; –ve, negative

[b] Supernatant of centrifuged 9,000 × g liver homogenate, containing the microsome and cytosol fractions, derived from rats previously treated with Aroclor to induce microsomal enzyme activity

[c] Originally reported in mol/l

[d] Estimated from chart

[e] 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide

8.4.2 Gene mutation *in vitro*

The mutagenicity of the chemicals of the cyanide family has been investigated in gene mutation assays in bacteria and mammalian cell lines using a variety of methods to induce metabolic activation (Table 50). Cytotoxicity has been observed at the highest test concentrations.

The studies of Hébert (1993), Monsanto (1983a) and Zeiger *et al* (1988) are considered to be the key studies for gene mutation in bacteria; for mammalian cells this is the HPRT test by Godek (1983).

Table 50: Gene mutation in vitro

Species, strain	Protocol	Metabolic activation	Concentration	Concentration as CN^-	Result [a]	Remark	Reference	CoR
Bacteria								
S. typhimurium, TA1537, TA1538, TA98 (frame shift mutation), TA1535, TA100 (base-pair substitutions)	Plate incorporation	+/– S9 [b]	Not stated (HCN)		**–ve**		Leuschner *et al*, 1983a cited by WHO, 1993	4c
S. typhimurium, TA1535, TA1537, TA1538, TA98, TA100	Plate incorporation	Aroclor-induced rat liver S9	6 - 495 µg ACH/plate	2 - 151 µg/plate	**–ve**	Toxic at high concentrations	Monsanto 1983a	1a
S. typhimurium, TA1535, TA97, TA98, TA100	Pre-incubation	Aroclor-induced rat and hamster liver S9	0.3 - 166 µg ACH/plate	0.1 - 51 µg/plate	**–ve**		Zeiger *et al*, 1988	1b
S. typhimurium, TA1535, TA97, TA98, TA100	Pre-incubation	Aroclor -nduced rat and hamster liver S9	0.3 - 333 µg NaCN/plate	0.2 - 177 µg/plate	**–ve**	Toxic at high concentrations	Hébert, 1993	1b
S. typhimurium, TA1530	Standard agar, pre-incubation	*S. typhimurium* DW 379 and 385, rat liver S9	6.5 µg NaCN/ml in pre-incubation mixture	2.6 µg/ml	**–ve**	Unusual pre-incubation protocol, only one strain; toxic metabolite	Owais *et al*, 1985	2e

Table 50: Gene mutation in vitro (cont'd)

Species, strain	Protocol	Metabolic activation	Concentration	Concentration as CN^-	Result [a]	Remarks	Reference	CoR
S. typhimurium, TA98, TA100	Plate incorporation	Aroclor-induced rat and hamster liver S9	0 - 40 µg HCN/plate	0 - 39 µg/plate	**+ve** (– S9)	HCN induced a slight increase in his^+ revertants in TA100, rate of spontaneous revertants not reported	Kushi *et al*, 1983	2e
S. typhimurium, TA97, TA102	Plate incorporation	Aroclor-induced rat liver S9	Not stated (KCN)	-	**–ve**		De Flora *et al*, 1984a,b	4e
S. typhimurium, TA1535, TA1537, TA1538, TA98, TA100	Plate incorporation	Aroclor-induced rat liver S9	195 µmol KCN/plate [c]	78 µg/plate	–ve	Toxic	De Flora, 1981	4e
S. typhimurium, TA1530, TA1535, TA1950	Plate incorporation	Not specified	Not stated ("wide range", KCN and NaCN)	-	**–ve**	No details reported	Kleinhofs and Smith, 1976	4e
Eucaryotic cells - fungi								
Neurospora crassa, (wild type)	Induction of biochemical mutants by cyanide treatment of conidia	None	5 mg KCN/l [d]	200 µg/ml	**+ve**	Increased number of unspecified biochemical mutants	Wagner *et al*, 1950	3c

Table 50: Gene mutations in vitro (cont'd)

Species, strain	Protocol	Metabolic activation	Concentration	Concentration as CN^-	Result [a]	Comment	Reference	CoR
Mammalian cells								
Chinese hamster cells, V79 (genetic marker HGPRT [e])	2 h	+S9	400, 800, 1,000, 2,000, 3,000 µg KCN/ml	160, 320, 400, 800, 1,200 µg/ml	**–ve**	KCN was tested up to a high cytotoxicity	Leuschner *et al*, 1989b cited by WHO, 1993	4c
Chinese hamster cells, V79 (genetic marker HGPRT)	24 h	–S9	1,000, 2,000, 3,000, 4,000, 6,000, 8,000, 10,000 µg KCN/ml	400, 800, 1,200, 1,600, 2,400, 3,200 µg/ml	–ve	KCN was tested up to a high cytotoxicity	Leuschner *et al*, 1989b cited by WHO, 1993	4c
Chinese hamster cell line CHO-K1-BH4	HPRT [f] forward mutation	Aroclor-induced rat liver S9	100 - 950 µg ACH/ml	31 - 290 µg/ml	**–ve**	Cytotoxic at 500 µg/ml and higher	Godek, 1983	1a
Mouse lymphoma L 5178Y cell line	Thymidine kinase forward mutation	Aroclor-induced rat liver S9	Not specified	-	**–ve**	Only negative test result cited, no detailed test data	Garberg *et al*, 1988	4e

[a] +ve, positive; –ve, negative

[b] Supernatant of centrifuged 9,000 × g liver homogenate, containing the microsome and cytosol fractions, derived from rats previously treated with Aroclor to induce microsomal enzyme activity

[c] Reported as 3,000 nmol/plate

[d] Reported as 0.05%

[e] Hypoxanthine-guanine phosphoribosyl transferase (converts guanine to guanine monophosphate)

[f] Hypoxanthine phosphoribosyl transferase (converts hypoxanthine to inosine monophosphate)

In one test in *Salmonella typhimurium* TA100, a slightly positive result was obtained (Kushi *et al*, 1983) that has not been reproduced in any one of the other tests. There was no indication that cyanide has the potential to induce gene mutations in bacterial or mammalian test systems.

Wagner *et al* (1950) isolated unspecified "biochemical mutants" from *Neurospora crassa* cultures treated with 0.05% KCN (200 µg CN^-/ml). Cytotoxicity was not determined. By cross-reading to other test systems described in Section 8.4.1, the concentration used can be expected to have exerted significant cytotoxic effects in this test system. Due to the unspecific nature of the test procedure, the genetic background of the observed mutations and, hence, the mechanism which may have induced them, are unclear.

An *in vitro* Ames test with HCN in *S. typhimurium* strains TA1537, TA1538 and TA98 for detection of frame shift mutation and TA1535 and TA100 for base-pair substitutions was performed with and without metabolic activation. There was no indication of mutagenic activity of HCN under these conditions (Leuschner *et al*, 1983a cited by WHO, 1993).

A gene mutation assay was conducted with KCN in cultured Chinese hamster V79 cells. The cells were exposed for 2 hours with and 24 hours without metabolic activation. The KCN levels employed were chosen following a preliminary toxicity experiment. KCN was tested up to a high cytotoxicity. Under the test conditions, KCN was not mutagenic in the V79 cells (Leuschner *et al*, 1989b cited by WHO, 1993).

8.4.3 Gene mutation *in vivo*

In a review of gene mutation assays of the crucifer *Arabidopsis thaliana*, the author reported no mutagenicity in plant seedlings treated up to 650 µg KCN/ml (260 µg CN^-/ml) (Redei, 1982) (Table 51). No further test details (range of concentrations tested, toxicity) have been reported in this paper.

Table 51: Gene mutation in vivo

Species, strain	Protocol	Metabolic activation	Concentration (µg KCN/l)	Concentration (µg CN^-/l)	Result a	Remark	Reference	CoR
Plants								
Arabidopsis thaliana (Cruciferae)	Seeds exposed to test material, emerging plants screened for mutations	Implicit	650 [b], highest concentration tested	260	**–ve**		Redei, 1982	2e

[a] –ve, negative
[b] Reported as 10 mmol/l,

8.4.4 Chromosome damage *in vitro* and *in vivo*

The induction of chromosome aberrations *in vitro* by KCN has been investigated by Umeda and Nishimura (1979 CoR 2e). They found a dose-dependent increase in structural chromosomal aberrations (gaps, breaks and exchanges) in cells of the cell line FM3A (derived from a C3H mouse mammary carcinoma) at concentrations between 0.32 and 1 mmol/l (0.1 - 2.6 µg/ml CN^-). A concentration of 2 mmol KCN/l was cytotoxic as indicated by the fact that no cells could be found in metaphase arrest.

Possible chromosomal damage by cyanide *in vivo* has been investigated in plants and rodents (Table 52).

KCN-induced chromosome aberrations in the lateral roots of *Vicia faba*. The rate of aberrations was clearly concentration-dependent and was influenced by the presence of oxygen. Under anoxic conditions (nitrogen atmosphere) the aberration rate was substantially lower. The authors attributed this effect to increased peroxide concentrations in the cells due to inhibition of the oxidase as well as peroxidase enzymes by cyanide. At 1 mmol KCN/l (26 µg CN^-/ml), the peroxidase and catalase activity in *V. faba* cells was inhibited by 100%. Hydrogen peroxide (5 mmol/l) in combination with 0.1 mmol KCN/l (3 µg CN^-/ml) induced chromosome aberrations at concentrations where the individual substances had no effect when tested alone (Kihlman, 1957).

In a rat bone marrow chromosome aberration assay, doses of 1.5, 5 and 15 mg ACH/kgbw (0.15, 0.46, 4.6 mg CN^-/kgbw) were administered together with appropriate negative and positive controls. There were no treatment-related increases in the frequency of chromosomal aberrations. Under the conditions of the study, no significant difference was observed between the negative control and test groups when comparing modal chromosome numbers or mitotic index. The dose range had been based on available acute toxicity data and a range finding study. There was no indication of cytogenetic effects of ACH *in vivo* (Monsanto, 1983b). This test is in full compliance with current mutagenicity test guidelines and is considered to be the key study for this endpoint.

Chinese hamsters were orally dosed with HCN and preparations of metaphase cells were studied for structural chromosome aberrations. The incidence of aberrations or gaps was within the spontaneous range. Neither multiple aberrations nor pulverised metaphases were found. There was no indication of mutagenic properties relative to structural chromatid or chromosome damage *in vivo* (Leuschner *et al*, 1983b cited by WHO, 1993).

Table 52: Chromosome aberrations in vivo

Species, strain	Protocol	Dose	Dose as CN^-	Result [a]	Remark	Reference	CoR
Plants							
Bean, *Vicia faba* (lateral roots)	6 - 30 h preparation intervals, tested under aerobic and anoxic conditions	0, 0.1 - 1 mmol KCN/l	0, 3 - 26 µg/ml	+ve	Concentration-dependent increase of aberrations, especially under aerobic conditions	Kihlman, 1957	2e
Rodents							
Chinese hamster	Structural aberrations	0.4 mg HCN/kgbw, oral	0.39 mg/kgbw	**–ve**	After 6, 24 and 48 h	Leuschner *et al*, 1983b cited by WHO, 1993	4c
Rat, Sprague-Dawley, CD, 6 males, 6 females per dose and preparation	Bone marrow sampled at 6, 12, 24 and 48 h	1.5, 5, 15 mg ACH/kgbw, single oral dose	0.15, 0.46, 4.6 mg/kgbw	**–ve**	Fully valid study, exceeding the guideline requirements	Monsanto, 1983b	1a

[a] –ve, negative

8.4.5 Aneuploidy

Osgood and Sterling (1991) investigated the induction of aneuploidy, mating two strains of fruit flies (*Drosophila melanogaster*) with a specific genotype (females: y z/y² z f.YL; spa^{pol}; males: attached-X-Y, w; net). Genetic changes caused by non-disjunction were evidenced by changes in the eye colour of the F_1 generation, which normally resembles the wild-type. NaCN induced an increase in aneuploidy after exposures of 15 and 45 minutes at a near lethal dose specified as 0.2 mg/l. In the 45-minute exposure group 30 to 40% of the test flies died, the rest were partially immobilised for several hours. The authors assumed that the damage may have been caused by the well established drop of the intracellular ATP levels caused by cyanide, which may disrupt the chromatid separation.

8.4.6 Summary and evaluation

Valid data are available for all genetic endpoints. Under the test conditions of the standard mutagenicity tests there was no indication of mutagenic or genotoxic activity of cyanide.

Three of the genotoxicity tests (De Flora *et al*, 1984a,b; Bhattacharya and Rao, 1997; Vock *et al*, 1998) and the aneuploidy test in *Drosophila* by Osgood and Sterling (1991) indicate that metabolic failure at overtly cytotoxic or lethal cyanide concentrations may also affect DNA replication and chromatid separation. This is considered to be a threshold effect, which is not expected to occur at lower cyanide concentrations which do not inhibit the oxygen-dependent metabolism. These results have all been obtained between 1 and 10 mmol CN^-/l. It should be noted that a dose of just 0.1 mmol/kgbw is lethal to rodents (Section 8.1.1).

8.5 Chronic toxicity and carcinogenicity

There are no specific long-term studies conducted in accordance with OECD guidelines on the possible chronic or carcinogenic effects of HCN or other cyanides.

Caworth Farms rats (10/sex/group) were fed diets fumigated with HCN for 2 years. The concentration of HCN in the diets was determined analytically. Special jars were used in order to limit volatilisation of HCN from the food. The food was changed every two days. The dietary concentrations were 100 and 300 mg/kg on the first of 2 days and decreased to about 50 and 80 ppm respectively on the second day. In a preliminary study, 300 mg/kg had been identified as the maximum level that did not result in immediately decreased food intake. The animals did not accept higher concentrations due to reduced palatability. Survival, body-weight gain, food intake, and clinical behaviour were not different between treated and control groups in the two year

study. Absolute and relative organ weights (organs examined: liver, kidney, spleen, brain, heart, adrenals, testes and ovaries) were also comparable to controls. No treatment-related histopathological changes were observed in the organs examined (heart, lung, liver, spleen, gastro-intestinal tract, kidneys, adrenals, thyroid, testes, uterus, ovaries, cerebrum, cerebellum and brain). The NOAEL in this study was approximately 8.2 mg HCN/kgbw/d (7.9 mg CN^-/kgbw/d) (Howard and Hanzal, 1955) (Table 53).

Table 53: Chronic toxicity in rats fed on HCN fumigated diet

Strain, number/group, sex	Dose (mg HCN/kgbw)	Dose (mg CN^- /kgbw)	Frequency of treatment	Duration	Result	Remark	Reference	CoR
Carworth Farms, 10 M, 10 F	≈ 0, 3.2 or 8.2 [a]	0, 3.1, 7.9	*Ad libitum*	2 y	No treatment-related effects. NOAEL = 7.9 mg CN^-/kgbw/d	Initial concentration in diet of 100 and 300 mg/kg dropped to ≈ 50 and 80 mg/kg during the 2-d period between food changes	Howard and Hanzal, 1955	2e

[a] Calculated from reported food concentrations of average 75 (50 -100), 190 (80 - 300) mg/kg food, assuming average bw ≈ 400 g/rat (from study) and daily food consumption ≈ 17.2 g food (from study)

8.5.1 Evaluation

From the available data there is no concern for genotoxicity. There are no reliable data on carcinogenicity in animals. However, because of the steep dose-response relationship, it would, from a practical perspective, be extremely challenging to conduct a long-term study whilst maintaining near toxic levels of CN. In a limited 2-year dietary study in rats there were no indications of carcinogenicity in animals fed with diets fumigated with maximum tolerated doses of HCN.

The possibility for an indirect (non-genotoxic) tumorigenic effect of thiocyanate on the thyroid cannot be ruled out. However, populations displaying goitre as a result of consuming dietary cyanogens (cassava) have not been linked with a concern for cancer. Chronic stimulation of the thyroid in the case of a chronically elevated serum thiocyanate level, and in particular when in combination with iodine deficiency, could theoretically also lead to a formation of thyroid tumours. Competitive inhibition of iodine uptake into the thyroid by thiocyanate leads to a reduced formation of thyroid hormones (T3 and T4). Via a negative feed-back mechanism, this triggers the production of TRH that, in turn, triggers production of TSH. If the TSH level is chronically elevated, thyroid cell hypertrophy and hyperplasia may result. This may ultimately lead to some potential for neoplasia (Hill *et al*, 1998). Under conditions where thyroid-pituitary homeostasis is maintained, the steps leading to possible tumour formation are not expected to develop and the chances of tumour formation are negligible (Hill *et al*, 1998). Thus if exposure to levels leading to goitre formation are avoided, there should also be no risk of tumour formation. This effect has been observed in rodent studies (Hard, 1998), but in human epidemiological studies, even in areas with endemic goitre and cretinism, no increase in thyroid tumour prevalence was observed indicating that humans are less sensitive than rodents for that endpoint. This apparent difference between rodents and humans has been linked to the lack of a high affinity protein binding T4 in rodents and a higher turnover of T3 and T4 combined with a higher thyroid hormone synthetic activity in thyroid follicles. The accelerated production of thyroid hormones in rats is driven by serum TSH levels about 6 to 60 fold higher than in humans. The rodent thyroid, being more stimulated due to the higher thyroid hormone turnover, is more likely to develop goitre and neoplastic changes than the human gland (Hill *et al*, 1998).

8.6 Reproductive toxicity

8.6.1 Oral

HCN

Albino Carworth Farms rats (10/sex/group) were fed diets fumigated with HCN for 2 years. The concentration of HCN in the diets was analytically determined. Special jars were used in order to limit volatilisation of HCN from the food (changed every 2 d). The dietary concentrations were 100 and 300 mg/kg on the first of every 2 days and decreased to approximately 50 and 80 mg/kg, respectively, on the second day. (In a pre-study, 300 mg/kg had been identified as the maximum level that did not result immediately in decreased food intake. The animals did not accept higher concentrations due to reduced palatability.) There was no difference in survival, body-weight gain, food intake or clinical behaviour between treated rats and controls. No treatment-related effects on reproductive organs were identified as indicated by absolute and relative organ weights for testes and ovaries, and histopathology of the testes, uterus or ovaries. Other organs examined (heart, lung, liver, spleen, gastro-intestinal tract, kidneys, adrenals, thyroid, cerebrum, cerebellum and brain) were also unaffected. The NOAEL in this study was approximately 8.2 mg HCN/kgbw/d (7.9 mg CN^-/kgbw/d) (Howard and Hanzal, 1955).

The effect of diet, reproductive performance and postnatal development was studied in albino rats (10 females/group, strain not specified) fed *ad libitum* five diets consisting of (i) laboratory animal diet (LAD) (controls), (ii) a paste of 50% LAD mixed with gari (a form of cassava meal) containing 0.11 g HCN/kg (equivalent to 106 mg CN^-/kg) in oil, (iii) raw cassava *(Manihot utilissima*) containing 0.3.6 to 25 g HCN/kg (3,470 - 24,100 mg CN^-/kg), (iv) LAD containing 50 g KCN/kg (19,980 g CN^-/kg), or (v) LAD containing 100 g KCN/kg (39,950 mg CN^-/kg). Food consumption was not reported. Animals studied for dietary adequacy were fed for 14 weeks before being killed and organ weights and general haematological parameters measured. Animals studied for reproductive performance were fed for 2 weeks and mated with 5 male rats fed on normal diet. Mated females were kept on diet till full-term, at which point litter size and weight were recorded. Animals studied for postnatal development were taken from litters as above (in the case of normal diet and 50% gari) or from control litters (in the case of raw cassava and KCN-supplemented LAD) and allowed to suckle for 21 days from their dams (fed on diet) before being weaned onto diet. After a further 4 weeks, the young were killed and organ weights and general haemato- or histological parameters measured. Rats fed on LAD mixed with gari had increased serum thiocyanate levels but there was no corresponding effect on thyroid weight. Haematological and biochemical parameters also showed no effect of diet. Raw cassava was inadequate as a dietary replacement as indicated by loss of body weight, goitre, atrophy of the spleen and thymus, hair loss, apathy, anxiety, ever-readiness to bite and reduced haematological and biochemical parameters and an increase in incidence of cannibalism.

Rats fed with the KCN supplemented LAD diets also showed a marked dose-related body-weight loss, goitre, atrophy of the spleen and thymus, hair loss, apathy, anxiety and ever-readiness to bite, but also displayed necrosis of the liver, and tumours of the caecum and colon. Reproductive performance of animals fed on LAD with gari was comparable to controls. Body-weight gain during pregnancy was significantly reduced compared with LAD fed controls. Reproductive performance was significantly reduced in those rats fed raw cassava. None of the animals fed the KCN supplemented diets became pregnant. Litter size of LAD with gari fed rats was comparable to that of controls, but the average pup-weight was significantly reduced. According to the authors, this suggested that, although nutritionally adequate in normal adult rats, the 50% gari diet was inadequate during pregnancy. Litter size and average pup-weight was reduced with the raw cassava fed animals and this corresponded with an increased incidence of mothers eating their young. Young being weaned by mothers fed with KCN-supplemented diet had died by the end of the third week. Reduced pup-weight gain compared with LAD controls was observed for animals on gari and, to a greater extent, on raw cassava. This reflected the nutritional inadequacy of these diets during neonatal development (Olusi *et al*, 1979). The studies with gari are of no significance to this review as the gari used contained only trace amounts of cyanide, presumably because much of the cyanide is removed during processing. The studies with raw cassava are of questionable significance since, although the diet contained cyanide, it was nutritionally inadequate. Hence, it cannot be excluded that the effects observed were due to the advanced state of emaciation and not solely attributable to the presence of cyanide in the diet. LAD with 19.98 or 39.95 g CN^-/kg diet was overtly toxic to adult female rats and resulted in cessation of reproductive performance. These overtly toxic doses were also toxic to the developing neonate.

In a developmental study, female, mated Yorkshire gilts (pigs) (6/group) were fed *ad libitum* until parturition on a diet of fresh sweet cassava (low cyanide) with protein supplement and 0, 250 or 500 mg CN^-/kg added as KCN. The concentrations in the food were measured and reported as 30.3, 276.6 and 520.7 mg CN^-/kg diet, respectively. The feeding regimen consisted of 1.2 kg cassava plus 0.62 kg protein given in the morning and 2.1 kg of fresh cassava in the afternoon. Actual food consumption was not reported. On day 110 of gestation, 2 animals per group were killed. The remaining gilts remained on treatment until parturition, at which point they were changed to a standard diet throughout the 56-day lactation period. Dietary cyanide levels had no effect on the number or weights of 110-day old foetuses. Serum thiocyanate levels were slightly raised in the dams at 520.7 mg CN^-/kg and this increase was statistically different in the case of their foetuses. A small increase in maternal thyroid weights was observed with increasing level of cyanide, but this did not gain statistical significance. Histopathology revealed proliferation of glomerular cells of the kidney in gilts in all groups and reduced activity of the thyroid gland in gilts at 520.7 mg/kg. Cyanide fed to gilts during gestation did not affect performance during lactation. Milk thiocyanate and colostrum iodine levels were significantly higher at 520.7 mg/kg. Cyanide up to 520.7 mg CN^-/kg in the diet had no effect on the development and subsequent lactation of young pigs despite the fact that that the placental barrier

had been breached, as indicated by elevated levels of serum thiocyanate in the foetuses, and toxicity being observed in the thyroid and, perhaps, kidneys of the dams (Tewe and Maner, 1981).

ACH

In a teratogenicity study, pregnant Sprague-Dawley rats (25/group) were dosed on days 6 to 15 of gestation with 0, 1, 3 or 10 mg ACH/kgbw (corresponding to 0, 0.31, 0.92 and 3.06 mg CN^-/kgbw). Dose selection was made on the basis of marked maternal toxicity observed at higher doses observed in a range-finding study (Monsanto, 1983a). No deaths were observed during the exposure period. Maternal toxicity was evident by slight reductions in body-weight gain in mid and high exposure groups. Statistically significant differences between the high-dose group and controls were observed for the number (per dam) of corpora lutea and number of implantations. Numbers (per dam) of viable foetuses, post-implantation losses, mean foetal body weight and foetal sex distribution at all doses were comparable to controls. The incidence of foetal malformations and developmental variations for all foetuses of treated rats were also comparable to controls (Monsanto, 1983c,d). It may be concluded that 3.07 mg CN^-/kgbw was not teratogenic in the rat in the presence of maternal toxicity.

NaCN and KCN

The effect of a balanced diet containing NaCN was studied in growing dogs (6/group; age, sex and species not specified) for 14 weeks (Kamalu, 1993). Three diets containing (i) rice (control), (ii) gari (prepared cassava) expected to release 10.8 mg HCN/kg food (10.4 mg CN^-/kg) or (iii) rice containing NaCN (also expected to release 10.8 mg HCN/kg food) were fed at a rate of 100 g diet/kgbw. Plasma electrolytes, serum proteins, plasma-free amino acids, plasma enzymes and urine protein were measured. The histopathology of liver, kidney, myocardium, testis and adrenal glands were also studied. Diets of gari and rice with NaCN caused elevated plasma thiocyanate and haemato- or histological evidence of toxicity in the organs studied. In the animals fed gari there were occasional abnormal germ cells in the seminiferous tubules and occasional seminiferous tubules denuded to basement membrane of germ cells but with remnants of Sertoli cells. Spermatogenesis, however, appeared to be normal as indicated by the percentage of round tubules in stage 8 of the spermatogenic cycle. The diet supplemented with rice and NaCN caused nephrosis and a significant reduction in relative testicular germ cells in stage 8 of the spermatogenic cycle. There was also marked testicular germ cell sloughing and degeneration. The more marked testicular toxicity observed in animals fed with the rice and NaCN diet may be the result of higher levels of CN being released from this diet, as significantly higher urinary and plasma thiocyanate levels were measured in these animals. Cyanide at 10.8 mg CN^-/kg in the diet

(equivalent to 10.4 mg CN^-/kgbw/d) caused testicular toxicity in the growing dog in the presence of toxicity in the liver, kidney, myocardium and adrenal glands.

F344 rats and $B6C3F_1$ mice (10/sex/species/group) received 0 (controls), 3, 10, 100 or 300 mg/l NaCN in the drinking water for 13 weeks. In addition to the normal investigations, vaginal cytology was examined 12 days prior to killing, and sperm motility was evaluated at necropsy. Water consumption was reduced at 100 and 300 mg/l. Corresponding decreases in urine excretion and increases in urine specific gravity were reported in rats. Increased urinary thiocyanate levels were observed at 10 to 300 mg/l. A slight decrease in body-weight gain was observed in male rats and a slight decrease in final mean body weight in female mice exposed to 300 mg/l NaCN, but no such decrease was seen in female rats or male mice. No other treatment-related clinical signs were observed. No treatment-related changes in haematology or clinical chemistry, and gross or microscopic histopathology were observed in any treatment groups. The left and caudal epididymal weights of all male rats and the 300 mg/l male mice were significantly lower than those of the controls. In rats receiving 300 mg/l NaCN, the left epididymal and testis weights and the number of spermatid heads per testis were also lower than in the controls. Sperm motility in all exposed male rats was less than in the controls, but the changes were not considered biologically significant by the authors and were in the range of normal values reported from different laboratories. In mice, no changes in sperm motility were observed. Pro-oestrus and di-oestrus time was prolonged in female rats of the 100 and 300 mg/l groups compared to controls, without a clear dose-related response. According to the authors these effects cannot unequivocally be attributed to cyanide exposure. No such effects occurred in female mice. A NOAEL of 100 mg/l for mice (equivalent to 8.6 and 9.6 mg CN^-/kgbw/d in male and female mice, respectively) can be derived from this study. No clear NOAEL can be derived for rats due to the unclear significance of the findings concerning the reproductive organs (Hébert, 1993).

In a 13-week toxicity study male Sprague-Dawley rats (≈ 30/group) were administered KCN in the drinking water at dose levels of 40, 80 and 160/140 mg KCN/kgbw/d. Testes weights and histopathology was evaluated and no effects were observed (Leuschner *et al*, 1989a cited by WHO, 1993).

The developmental toxicity of KCN and potassium thiocyanate was investigated in pregnant Wistar rats (20 females/group). The rats received daily doses of 0, 1, 3 or 30 mg KCN/kgbw, or 0, 0.8, 2.4 or 24 mg KSCN/kgbw in the drinking water between day 6 and 20 of gestation. Animals were killed on day 20 of gestation (trial A) or one day after weaning (trial B). Foetuses were analysed for skeletal and visceral anomalies (trial A). Samples of blood and different organs (kidney, pancreas, brain, thyroid, spleen, lungs and liver) were collected from dams (trial A and B) and weaned pups (trial B) for biochemical and histopathological evaluation. Results showed high thiocyanate levels in dams at all dose levels in both trials. The intensity of the histological lesions observed in dams of trial B was similar to that of trial A. Exposure of dams to

oral doses of up to 30 mg KCN/kgbw/d or 24 mg KSCN/kgbw/d did not cause foetal malformations. While some histological effects were observed in the brain and liver of the offspring, these lesions were seen also in the dams, and at a higher incidence, supporting the conclusion that there was no specific sensitivity of the foetus to cyanide compounds (Sousa *et al*, 2007).

8.6.2 Inhalation

ACH

In a male fertility study, Sprague-Dawley rats (15 males/group) were exposed (6 h/d; 5 d/wk) on 48 exposure days, i.e. for 69 days in total, to concentrations of 0, 10.0, 28.5 or 57.2 ppm ACH (equivalent to 0, 3.1, 8.7 or 17.5 ppm CN^-; 0, 10.8, 30.8, 61.9 mg CN^-/m^3). After the treatment period, each male was mated consecutively with 3 untreated females. There were no effects of treatment on males as indicated by mortality, clinical observations and necropsy observations (males were killed about 3 weeks after the end of the exposure period). Only at 57.2 ppm did the rats show lower mean body weights but the difference was not statistically significant from controls. The number of live implants and pre- and post-implantation losses were comparable for females mated with untreated or treated males. The authors concluded that exposure to 57.2 ppm ACH (equivalent to 8.4 mg CN^-/kgbw/d [a]) did not exhibit reproductive toxicity in male rats (Monsanto, 1985a). The highest exposure studied can be assumed as the NOEL for male reproductive effects, although no effect level (LOEL) was identified in this study.

In a female fertility study, Sprague-Dawley rats (24 females/group) were exposed (6 h/d, 7 d/wk) by inhalation for 21 days to 0, 10.7, 30.4 or 58.6 ppm ACH (equivalent to 0, 3.3, 9.3 or 17.9 ppm CN^-; 0, 11.6, 32.9, 63.4 mg CN^-/m^3). There was no indication of treatment-related effects on body weight during exposure or during gestation. After the exposure period, the females were mated with untreated males. At examination on gestational day 13 to 15, the fertility of mated females was comparable between treated groups and the control group for mating efficiency, pregnancy rates, number of live implants and pre- and post-implantation losses. The authors concluded that exposure to 58.6 ppm ACH (equivalent to 8.6 mg CN^-/kgbw/d [a]) did not have any effects on fertility of female rats (Monsanto, 1985b). The highest exposure studied can be assumed as the NOEL for female reproductive effects, although no effect level (LOEL) was identified in this study.

[a] Dose calculated using minute volume of 150 ml/min and average bw of 200 g/rat (ECB, 2003 update defaults), assuming 50% absorption

8.6.3 Other routes

Pregnant golden hamsters (10/group) were continuously infused *s.c.* (using osmotic mini pumps) with NaCN at doses of 0, 0.126, 0.1275 or 0.1295 mmol NaCN/kgbw/h (0, 6.18, 6.25, 6.35 mg NaCN/kgbw/h; 0, 3.28, 3.32, 3.37 mg CN^-/kgbw/h) from days 6 to 9 of gestation. The total dose was equivalent to 30 to 40 times the acute *s.c.* LD_{50}, indicating the considerable capacity of the animals to detoxify cyanide. Doses greater than 3.28 mg CN^-/kgbw/h produced high incidences of malformations and resorptions. (In a preliminary study, 0.133 mmol/kgbw/h [6.52 mg NaCN/kgbw/h; 3.46 mg CN^-/kgbw/h] caused 100% foetal mortality and some maternal deaths.) Malformations included non-closure of the neural tube, exencephaly, encephaly and microphthalma. At the highest dose studied (3.37 mg CN^-/kgbw/h), obvious signs of toxicity were apparent in all the dams. A statistical analysis of the incidence of malformations in surviving foetuses did not correlate with the overall incidence of signs of overt toxicity in the dams. Contemporaneous infusion of thiosulphate protected the dams and foetuses from the toxic effects of cyanide (Doherty *et al*, 1982).

8.6.4 Summary

The available feeding studies using diets of cassava or dietary supplements of KCN/NaCN are of limited value, but do indicate that high concentrations of dietary cyanide can be toxic to male and female reproduction and cause toxicity to the developing foetus and neonate. However, this occurred only at concentrations that caused overt systemic toxicity. Studies using lower dietary concentrations were conflicting and inconclusive but some were suggestive of testicular toxicity. Doherty *et al* (1982) also suggested testicular toxicity and teratogenic potential in the absence of maternal toxicity. However, this was based upon a statistical analysis of only a small number of dams with surviving foetuses. The only studies that give a clear indication of the relative sensitivity of the reproductive system are those that have used ACH. ACH showed no evidence for teratogenic effects following gavage dosing in rats up to and including doses that produced maternal toxicity. In male and female fertility studies in rats, inhaled ACH showed no reproductive effects up to concentrations that were limited by the threshold to acute toxicity (local irritative effects and systemic lethality) as indicated in repeated-dose inhalation studies (Section 8.3.3). Therefore, although cyanide is overtly toxic to the reproductive system, there is no reason to suspect that this system is any more sensitive than other organ systems.

NOAELs of 8.6 and 9.6 mg CN^-/kgbw/d (100 mg NaCN/l) in male and female mice, respectively, can be derived from the drinking-water study conducted by Hébert (1993). This value is consistent with the NOEL of 7.9 mg CN^-/kgbw/d (8.2 mg HCN/kgbw/d) in male and female rats from the fumigated diet study of Howard and Hanzal (1955). It is also essentially the same as the NOEL of 8.4 and 8.6 mg CN^-/kgbw/d (57.2 and 58.6 ppm ACH) by inhalation in

male and female rats, respectively (Monsanto, 1985a,b) and provides some confidence that the slight effects observed by Hébert (1993) in male rats were unlikely to be cyanide related. This is also supported by the fact that no effects on the testes were seen in a comparable rat drinking-water study at dose levels up to 56 mg CN^-/kgbw (140 mg KCN/kgbw) (Leuschner *et al*, 1989a cited by WHO, 1993).

8.7 Special studies

8.7.1 Co-exposure to cyanide and noise

Long Evans pigmented rats (6 - 16 males/group; 2 - 3 months old) were exposed (nose-only) to HCN (0, 10, 30 or 50 ppm; 0, 11.2, 33.7, 56.2 mg/m^3) for 3.5 hours, noise alone (in inhalation chamber) for 2 hours, or noise for 2 hours and HCN (10, 30 or 50 ppm) for 3.5 hours. Background noise in the exposure chambers was below 35 dB for all octave bands having a centre frequency (geometric mean) of 2 kHz and higher. Broadband noise (100 dB) with a centre frequency of 13.6 kHz was applied, resulting in a preferential disruption of high frequency auditory function while leaving low frequency auditory function intact. Four weeks after exposure, 'pure tone compound action potential' thresholds were determined by placing electrodes onto the round window of the cochlea and the neck muscle (reference electrode) of anaesthetised rats and measuring the action potentials in response to noise. The sound level necessary to generate a visually (oscilloscope) detectable action potential (amplitude ≈ 1 μV) was determined for pure tones of 2, 4, 6, 8, 12, 16, 20, 24, 30, 35 and 40 kHz applied for 10 ms (9.7 ×/s, rise/fall within 1 ms). Histology of the cochlea was performed in 3 controls, 4 rats exposed to noise only and 3 rats each exposed to 10 or 30 ppm of HCN.

Action potential thresholds of animals receiving 10 and 30 ppm HCN without additional noise exposure were comparable to those of controls. The rats at 50 ppm HCN had slightly elevated thresholds (10 dB) at the highest and lowest frequencies; the difference was not statistically significant. Noise alone induced a significant threshold elevation (12 dB) between 12 and 40 kHz, but not between 2 and 8 kHz. HCN combined with noise resulted in a dose related impairment of action potential thresholds (i.e. hearing loss), compared to noise only, that was significant at 30 and 50 ppm. Losses were 24 and 36 dB, respectively, between 12 and 40 kHz, and 20 dB at 50 ppm and 8 kHz. At 10 ppm HCN plus noise, there was 15 dB impairment between 12 and 40 kHz.

Benchmark dose analysis showed a linear relationship for (i) 10% hearing loss beyond the effect of noise alone (1 dB loss) in the frequency range of 12 to 40 kHz, resulting in a benchmark dose of 3 ppm with a lower bound confidence limit of 2 ppm, (ii) 5 dB hearing loss in the frequency ranges of 12 to 40 kHz, resulting in a benchmark dose of 11 ppm with a 95% lower confidence

limit of 9 ppm, and (iii) hearing loss corresponding to one standard deviation above the mean effect for noise alone, resulting in benchmark doses of 21 ppm (lower confidence limit of 16 ppm) at 12 to 40 kHz and 11 ppm (lower confidence limit of 9 ppm) at 20 to 40 kHz. Histological analysis of the cochleae, following location of hair cell damage using cytocochleograms, revealed that 10 and 30 ppm HCN in combination with noise had induced a slightly higher outer hair cell loss, compared to controls, mainly at locations corresponding to frequencies higher than 20 kHz. Hair cell losses were 5% in noise only rats, 6% (not significant) with 10 ppm HCN plus noise, and 9% (significant) at 30 ppm HCN plus noise. HCN exposure alone had no effect on cochleal hair cells.

In conclusion, 30 ppm HCN exposure for 3.5 hours combined with noise (100 dB, centre frequency 13.6 kHz) for 2 hours significantly increased hearing loss and loss of cochleal hair cells in rats. A benchmark dose of around 10 ppm HCN combined with noise levels of 100 dB was derived for the increase of noise-induced hearing loss (Fechter *et al*, 2002).

8.7.2 Synergism of cyanide and carbon monoxide

It has been observed that fire victims sometimes die without reason, in spite of their cyanide and CO blood levels being sublethal. A synergistic mode of action has been suspected.

The effects of co-administration of cyanide and CO were studied in ICR mice (5 males/group) injected *i.p.* with 4 to 9 mg KCN/kgbw following prior exposure to atmospheres containing 0.63 to 0.66% CO or air alone (control) for 3 minutes. A significantly lower LD_{50} for KCN was observed in CO pretreated animals compared to air controls (Norris *et al*, 1986).

In another series of experiments, ICR mice were pretreated *i.p.* with 1 to 6.35 mg KCN/kgbw or saline (control) and then exposed to CO (0.325 - 0.375%) for 40 minutes. Of the saline controls, 10 to 20% died at the end of the 40-minute exposure period. A dose of 6.35 mg KCN/kgbw (LD_{10}) produced 100% lethality. The highest dose without a significant increase in lethality compared to saline controls was 3.0 mg KCN/kgbw. Time of death was not different between treated animals and controls. At the end of the exposure period, CO and cyanide blood levels were not different between groups, suggesting that the effect was caused by synergism of CO and cyanide. The authors postulated that the synergism could be due to augmented inhibition of cytochrome *c* oxidase in the CNS by both substances (Norris *et al*, 1986).

9. EFFECTS ON HUMANS

9.1 Acute toxicity

It is generally recognised that there is a considerable variability in regard to 'lethal doses' reported for cyanide in the literature and that this is probably due to differences in supportive care and therapy rendered during treatment. For adults, the potentially lethal oral dose of cyanide salts in the absence of medical care (antidote treatment) is between 200 and 300 mg (Bonsall, 1984).

Oral ingestion of high doses of cyanide, or inhalation exposure to high concentration of cyanide vapour, is rapidly followed by systemic poisoning, convulsions, collapse, coma and death. After inhalation of HCN at low concentrations, ingestion of small amounts of cyanide salts, or contamination of the skin, the following signs and symptoms may develop: anxiety and excitement, rapid breathing, faintness, weakness, headache (pulsating), constricting sensations in the chest, facial flushing, dyspnoea, nausea and vomiting, diarrhoea, dizziness, drowsiness, confusion, convulsions, incontinence of urine and faeces, coma and respiratory irregularities (Vogel *et al*, 1981; Pontal *et al*, 1982; Bismuth *et al*, 1984; all cited by Ballantyne, 1987a). An early characteristic feature of acute cyanide poisoning, particularly with smaller doses, is the development of tachypnoea and hyperpnoea, resulting in increased tidal volume. This may clearly enhance the uptake of HCN in the early stages of respiratory exposure to the vapour (Ballantyne, 1987a).

For ACH there are no data available from controlled human studies. Anecdotal case reports of incidents within Task Force member companies indicate that ACH is acutely extremely toxic by the inhalation, dermal and oral routes, although there is no documentation of effects relative to dose. Due to the low vapour pressure of ACH, inhalation exposure is predominantly to HCN, the decomposition product.

9.1.1 Oral

Gettler and Baine (1938) reported 4 cases of lethal cyanide poisoning. They calculated the absorbed dose from the combined tissue levels and the total dose from combined tissue levels plus unabsorbed amount in the stomach. From that, the authors estimated absorption rates, after oral ingestion, of 10 to 81% and lethal absorbed doses between 0.5 and 3 mg CN^-/kgbw, while the external doses ranged between 0.4 and 23 mg/kgbw. The minimum lethal absorbed dose was 0.5 mg CN^-/kgbw, and the average fatal dose 1.4 mg CN^-/kgbw.

9.1.2 Dermal

The LD_{50} for HCN by the dermal absorption route has been estimated at about 100 mg/kgbw (Rieders, 1971).

Murray *et al* (1987) reported 27 cases of cyanide poisoning registered in the UK from 1963 to 1984 upon skin contact (one case due to organic cyanide), eye contact or combined oral and skin exposure. No further details are available. Apparently there were no fatalities.

NIOSH (1976) reviewed several earlier cases of cyanide poisoning following skin contact as follows.

> "In 1905, McKelway [cited by NIOSH, 1976] reported a case of a 38 year old female hairdresser, who used a hickory-nut-sized piece of KCN to remove stains from a silver-containing dye from her fingers. She moistened her fingers and hands and rubbed them vigorously with the lump of KCN for 5 to 10 minutes, thus removing the stain. Before washing her hands, she experienced vertigo, then screamed and fainted. Her husband revived her in fresh air. Later that evening she vomited, and a physician was called. He found her in a state of shock and that her lips, fingers, and hands were deeply cyanosed. She was treated for shock and recovered in 3 days.
>
> In 1908, Nolan [cited by NIOSH, 1976] reported a case of dermatitis in a worker engaged in scrubbing zinc shavings in the KCN 'clean up' in the cyaniding process of gold reduction. In this process, the auriferous ore is crushed into a fine aggregate through which a 0.5% KCN solution is passed. This solution then passes into boxes filled with zinc shavings onto which the gold is precipitated from the auro-potassic cyanide in solution. The man's hands and arms were in close contact with this solution. He experienced an itching sensation immediately upon immersion of his hands. Scarlet specks soon appeared on the skin in contact areas and grew larger with time. They coalesced to form a large scarlet area with darker scarlet papules where the original lesions had been. They itched and burned for about 2 hours and the redness began to disappear after 12 hours. Slight giddiness and headache were the only systemic symptoms noted.
>
> Collins and Martland [cited by NIOSH, 1976] in 1908 reported that cyanide was absorbed through the skin of a 38 year old hotel worker who polished silver for 2 years by placing it by hand in a KCN solution and then drying it. His hands and arms became brownish-red, his fingernails were black, and he had distressing itching, diarrhoea, headache, pain and stiffness in the back and neck, weakness in the arms and legs, and retention of urine. He eventually developed clinical manifestations resembling those of acute anterior

poliomyelitis. He remained incapacitated during 6 months of treatment and then gradually responded to electrical treatment to the point where he could walk without braces and crutches. Whether this paralysis should be attributed to cyanide is not clear.

The International Labour Office [cited by NIOSH, 1976] in 1930 summarised a report first presented by Bridge in 1923 of a fatal case of skin contact with unspecified inorganic cyanide. The skin of the victim had been removed in patches as if it had been burned.

Smith [cited by NIOSH, 1976] in 1932 reported a case of a gold plater who had worked at his trade for 20 years, 10 in a small shop plating rings and pins in small pans containing KCN, which he made up. When plating, he heated the solution to boiling, which probably resulted in splattering and caused HCN evolution. He had a rash on his hands, arms, and face, which he attributed to the cyanide solution. He also had abdominal pain, convulsions, weight loss, weakness, dizziness, muscular cramps, and vomiting. Some of these signs and symptoms could be attributed to skin absorption and some to inhalation of HCN and aerosolised cyanide salts.

In 1955, Tovo [cited by NIOSH, 1976] described a fish poacher who died from absorption of KCN through the skin. The poacher added KCN to a river upstream and others netted the trout downstream. He was found 3 hours later curled up on the side of the road and without his boots and stockings, which were found on the riverbank. He died later that day without recovering consciousness. Necropsy revealed brownish-red blotches from knee to instep that smelled of bitter almonds, as did the blood. The body had a violet hypostatic coloration. The mother of the man pointed out that the legs of his trousers had been rolled up above his knees and were dry but that those of his long cotton underwear were wet at the bottom. Chemical examination showed the presence of cyanide in the blood, urine, and several of the vital organs, but concentrations were not reported. Tovo suggested that the man had placed his stock of KCN temporarily in his boots to wade into the water and that, either by accident or by misjudging the depth of the water, he stepped into water deep enough to flow over the tops of his boots. Although he escaped from the river as quickly as possible and removed his boots and stockings, he did not have the caution to remove his long underwear soaked with a solution of KCN. Consequently the poison was able to continue to penetrate through the man's skin, more readily because of its corrosive activity, and eventually to cause his death.

In 1926, Raestrup [cited by NIOSH, 1976] described an accident in which a man was killed when the fused KCN that he was carrying spilled into a puddle of water. The boiling water-cyanide solution splashed in his face. He lost consciousness and died 3 hours later. A similar case was reported by Muller-Hess in 1932 [cited by NIOSH, 1976] in which a worker was struck on the head and shoulders with a splash of 80% NaCN and

> died in less than 1 hour. Several other similar cases reported by various authors were summarised by Tovo [cited by NIOSH, 1976] but most of these did not involve occupational poisonings."

Potter (1950) reported a case of a worker taking samples in a HCN chamber (process not specified) and wearing a fresh respirator. When experiencing some difficulties with obtaining the sample he removed one glove and spilled some liquid HCN onto his hand. He waived the hand to help HCN evaporate and put on the glove again. A few minutes later he came out of the chamber and washed the gloves before removing them. About 5 minutes after contaminating his hand, he experienced typical cyanide poisoning symptoms (dizziness, difficulty in breathing followed by unconsciousness). He was treated immediately and finally recovered completely.

Dry NaCN on intact dry skin did not result in absorption of cyanide in amounts sufficient to produce signs of systemic toxicity. According to Dugard (1987), the amount and speed of absorption through human skin is dependent on the size of the contaminated skin area, the duration of skin contact and the pH of the cyanide salt or cyanide salt solution (a low pH resulting in rapid absorption, a high pH in slow absorption).

After brief skin contact with strong alkaline cyanide salts, only burns due to alkalinity have been observed. Symptoms of systemic poisoning have not been reported.

Kolpakov and Prokhorenkov (1978) reported allergic dermatoses among 310 gold-recovery workers. The incidence was higher in winter. Judging from the abstract, the relation between dermatosis in humans and NaCN exposure remains unclear.

Jacobs (1984) reported an industrial accident caused by skin contact and working clothes contaminated with HCN. The worker involved did not have breathing protection and immediately lost consciousness. In a case like this, it does not seem to be possible to speculate about the fractions of HCN inhaled and absorbed through the skin.

Concentrations of 7,000 to 12,000 mg HCN/m^3 (6,230 - 10,680 ppm) were estimated to be fatal after 5 minutes exposure of workers with self-contained respirators without effective skin protection (Minkina, 1988 cited by IPCS, 2004).

9.1.3 Inhalation

Inhalation of 600 to 700 ppm HCN (674 - 787 mg/m^3) for 5 minutes or approximately 200 ppm (225 mg/m^3) for 30 minutes may be lethal (IPCS, 1993, 2004; US-EPA, 1988).

Barcroft (1931) exposed himself (70 kgbw) to 540 mg HCN/m^3 (481 ppm) for 1.5 minutes and survived without losing consciousness. He reported nausea 3.5 minutes after cessation of exposure and difficulties to concentrate after 10 minutes. Bonsall (1984) reported accidental exposure for 6 minutes to about 500 mg/m^3 (445 ppm). Although exposure caused unconsciousness and treatment with thiosulphate was only started after more than 1 hour, this exposure was finally without sequelae. The author stated that this was a rather untypical case and one would normally expect a concentration of 181 ppm to be lethal after 10 minutes. The author quoted typical exposure × time-responses (Table 54).

Table 54: Typical response following HCN inhalation after Bonsall (1984)

Concentration		Time (min)	Response
(ppm)	(mg/m^3) [a]		
270	303	0	Immediately lethal
181	203	10	Death
135	152	30	Death
110 - 135	124 - 152	30 - 60	Death
45 - 54	51 - 61	30 - 60	Tolerated without immediate or later effect
18 - 30	20 - 34	Several hours	Slight symptoms

[a] Converted values

Chataigner *et al* (1989) provided a list of cyanide levels in whole blood with the corresponding symptoms of poisoning (Table 55). The subjects included 26 workers exposed when cyanide-containing compounds were accidentally mixed with acid, a child that had inhaled HCN fumes during combustion of a polyurethane mattress, and another child intoxicated following fumigation of a hotel room.

Table 55: Blood levels of cyanide and symptoms after Chataigner *et al* (1989)

Concentration in whole blood		Symptoms
µg CN^-/l	µmol CN^-/l	
0 - 320	0 - 12	Absence of toxic signs, non-smokers
16 - 520	0.6 - 20	Absence of toxic signs, non-smokers
500 - 1,000	19 - 38	Slight intoxication, vasodilatation-tachycardia
1,000 - 2,500	38 - 96	Moderate intoxication, confusion, limited consciousness
2,500 - 3,000	96 - 115	Serious intoxication, coma
≥ 3,000	≥ 115	Potential lethal intoxication

9.1.4 Intravenous toxicity

Wexler *et al* (1947 cited by NIOSH, 1976) studied the effect of cyanide on the electrocardiogram of 16 normal soldiers. The men were injected *i.v.* with 0.11, 0.15 or 2 mg NaCN/kgbw (0.06 - 0.11 mg HCN/kgbw), varying with the amount needed to elicit respiration stimulation in different cases. Following NIOSH (1976), "The electrocardiogram of 15 of the 16 men revealed a sinus pause without evidence of auricular activity persisting for 0.88 to 4.2 seconds. This sinus pause immediately preceded or accompanied the respiratory stimulation. Immediately after the pause, there were marked sinus irregularity and a decreased heart rate, which persisted for periods ranging from a few seconds to 2 minutes. Heart rates then accelerated to above pre-injection rates. Heart rate and rhythm were generally restored within 3 minutes. The 16th subject failed to show a sinus pause and exhibited only a slight acceleration in heart rate. One of the subjects experienced what the authors described as a momentary dim-out during the test." These observations seem to demonstrate that cyanide has no specific action on the heart apart from those typical of hypoxia and anoxia.

9.1.5 Clinical features of cyanide poisoning

There are many sources in the literature that describe the general symptoms of cyanide poisoning. In general, there is no difference between the routes of exposure (Bryson 1987; Borron and Baud, 1996, for example). Early symptoms include anxiety, headache, vertigo, confusion and hyperpnoea followed by dyspnoea, cyanosis, hypotension, brachycranic and sinus or atrio-ventricular nodal arrhythmia. The secondary stage of poisoning impairs consciousness and causes coma and convulsions. The pulse becomes weaker and more rapid. Opisthotonos and trismus may be observed. Late signs include hypotension, complex arrhythmia, cardiovascular collapse, pulmonary oedema and death. The experience of the Toxicological Intensive Care Unit at

Fernand Widal Hospital in Paris was that 36 cases of inhalation or ingestion of cyanide or cyanogens, excluding smoke inhalation, showed a wide range of symptoms (Borron and Baud, 1996) (Table 56).

Table 56: Symptoms of cyanide poisoning (Borron and Baud, 1996)

Symptom	Frequency	% of total
Asymptomatic	8	22
Cardiovascular collapse	10	28
Coma	13	36
Convulsions	6	17
Metabolic acidosis	18	50
Post anoxic coma and death	5	14
Psychomotor retardation	6	17
Respiratory arrest	8	22

Clinical chemistry

Cyanide concentration in blood and plasma

The blood cyanide concentration is considered to be the gold standard in confirmation of acute cyanide poisoning. This has been demonstrated, for example, when cyanide blood levels were linked to clinical criteria predictive of cyanide intoxication in 138 smoke inhalation victims out of 464 admitted (Borron and Baud, 1996) (Table 57).

Table 57: Cyanide intoxication in 138 smoke inhalation victims (Borron and Baud, 1996)

Neurological disturbances [a]	Blood cyanide > 40 µmol/l	Blood cyanide < 40 µmol/l
Present	39	50
Absent	1	48

[a] Neurological disturbance defined as loss of consciousness or disturbance or alteration of superior functions (change in behaviour, psychomotor retardation).

Blood thiocyanate level

The determination of blood thiocyanate level is another indicator of cyanide poisoning (after some time).

Lactic acidosis / Hyperglycaemia

Anoxic metabolism due to cyanide intoxication results in lactic acidosis, accompanied by an increase in blood glucose (Section 8.0.2).

The immediate and serial measurement of plasma lactate concentration has been found to be useful in assessing the severity of cyanide poisoning. Eleven patients were studied. Before antidote treatment, the median plasma lactate concentration was 168 mg/dl (1,680 mg/l), the median blood cyanide concentration was 4.2 mg/l. Using the Spearman rank correlation test, there was a significant correlation between plasma lactate and blood cyanide concentrations. Before antidote treatment, plasma lactate concentration correlated positively with anion gap and inversely with systolic blood pressure, spontaneous respiratory rate and arterial pH (Baud *et al*, 2002).

9.1.6 Sensory and dermal irritation

HCN smells of bitter almonds. The odour threshold is 0.58 mg/m^3 (Table 1) and the smell is obvious at 10 mg/m^3. At higher levels, cyanide poisoning soon paralyses the nerves and the perception of smell disappears. A perception of irritation does not occur.

Contact dermatitis in connection with electroplating from metal cyanide solutions has been reported incidentally. One worker developed some scattered pustules on the back of the fingers of the right hand and erosions and crusting of the lower lip. Further, he developed a purplish-brown discolouration and lost of the free ends of his fingernails (partial onycholysis) on the right hand. These effects were caused by direct contact with the solution of gold KCN and disappeared after a period of 4 weeks (Budden and Wilkinson, 1978).

Two electroplating workers developed erythematous papules and excoriations, primarily follicular in distribution, over the dorsal surfaces of the hands and distal forearms. In addition, one of the workers experienced onycholysis (loss of finger nails). Patch testing for metals present in the plating solution (cobalt, nickel, copper and gold) was all negative. According to the authors, cyanide remained left as the only provocative agent for cyanide irritative dermatitis. In

many cases, the papular morphology had a common appearance. The follicular distribution suggested preferential follicular irritation by cyanide salts (Mathias, 1982).

Local irritant effects of cyanide on skin and eye occur only rarely. This is because the prevention of systemic effects via the dermal route by means of protective clothing precludes the occurrence of skin irritation.

9.1.7 Summary and evaluation

Cyanides are very toxic by the oral, dermal and inhalation route of exposure. After oral exposure the average lethal dose is 1.4 mg CN^-/kgbw. The dermal LD_{50} has been estimated to be about 100 mg/kgbw. Inhalation of 270 ppm HCN (303 mg/m^3) is immediately lethal, exposure to 110 to 135 ppm (124 - 152 mg/m^3) leads to death in 30 to 60 minutes. Blood cyanide levels of 2,500 to 3,000 µg CN^-/l of total blood were related to serious intoxication and coma. The onset of cyanide toxicity is normally very rapid (within seconds to minutes) in particular via the inhalation and oral routes. Early symptoms of cyanide intoxication include headache, vertigo and dyspnoea followed by coma and convulsions, and finally respiratory arrest, cardiovascular collapse and death. Local irritative contact dermatitis has been reported, particularly related to cyanide use in the electroplating industry.

9.2 Chronic toxicity

9.2.1 Occupational

Blanc *et al* (1985) reported their investigations of a silver reclaiming works where one worker had died. The study involved 36 former workers who had been exposed long-term to an excessive level of cyanide (15 ppm; estimated from measurements after closure of the plant). Additionally, skin contact with cyanide-containing solutions was reported and the possibility of ingestion due to eating at the workplace could not be excluded. The study involved physical examination, laboratory studies and a questionnaire to determine levels of exposure, symptoms during employment and current symptoms. Physical examinations found no palpable thyroid gland abnormalities, mucosal erosion or focal neurological deficit. Questionnaire data showed that, during the time of active employment, there was a high prevalence of non-specific symptoms. These included some symptoms (such as dyspnoea, syncope, chest pain and easy fatigue) that were consistent with acute cyanide poisoning. The prevalence of cyanide related symptoms increased significantly with level of exposure, supporting a dose response effect. The authors claimed that other symptoms (like headache, bitter almond taste and rash) that persisted seven or more months after exposure were also related to cyanide exposure. (The Task Force considered

that it is not possible to draw conclusions from those unspecific observations.) With regard to the clinical chemistry findings, the authors reported that the overall mean vitamin B_{12} level of the exposed group was below that of their laboratory mean. (The standard deviation reported was very high and only 3 individuals had vitamin B_{12} levels below the lower limit of the normal laboratory data. Furthermore, all vitamin B_{12} levels reported in the study were in the normal range of 240 to 900 pg/ml [Zins and Trinh, 2003].) Individual thyroid hormone levels (T3, T4, TSH) of the exposed individuals were all in the normal range, but the authors again reported a higher mean TSH value in the high exposure groups and related it to the exposure. Although the authors claimed to have observed indications of chronic cyanide poisoning in the studied workforce, the reported data do not suggest the presence of such effects. The only plausible explanation is that the symptoms were related to acute intoxications. Finally, the exposure situation was not well defined.

In a controlled study, 63 employees from a cyanide salt manufacturing plant were compared with 100 matched controls employed at a diphenyl oxide plant. Area concentrations (without personal protective equipment) ranged between 1 and 3 ppm HCN (1.1 - 3.4 mg/m^3) and personal monitoring levels, in different jobs, were between 0.01 and 3.6 mg/m^3 (0.009 - 3.2 ppm) (no individual measurements were reported). All subjects had full medical examinations and routine blood tests. In addition, blood samples were analysed for blood cyanide and carboxyhaemoglobin, and vitamin B_{12} and T4. In the well matched groups, blood cyanide levels, whilst still low, tended to be higher in cyanide workers and smokers. There were no differences in T4 or vitamin B_{12} levels between exposed individuals and controls. None of the workers had goitre. In the exposed workers, haemoglobin and mean corpuscular haemoglobin levels, white blood cell counts (including differential white blood cell analysis), other blood parameters and blood pressure were all within the normal range. The authors concluded that “cyanide salt manufacturing employees are as healthy as their non cyanide manufacturing colleagues” (Leeser *et al*, 1990).

Zdrojewicz *et al* (1985) examined 67 people in an electroplating plant of a car factory. The abstract reports that hypothyroidism was seen in 7 subjects. Tests with metapyrone and TSH determination showed that these subjects had primary hypothyroidism. No data were given on the exposure levels or duration of work.

The health of 36 male workers occupationally exposed to cyanide in three electroplating factories was studied and compared to 20 male controls of the same age and social group (non-smokers without exposure to chemicals). Breathing-zone air samples were collected by means of a midget impinger containing NaOH (0.1 mol/l) at a rate of 3 l/min for 15 minutes. The cyanide levels in the solutions were quantified by means of colorimetry. Ranges of cyanide (HCN) concentrations at the workplace were between 8 and 12.4 ppm in plant A, 4.2 and 8.8 ppm in plant B, and 5.9 and 9.6 ppm in plant C (9.0 - 13.9, 4.7 - 9.9 and 6.6 - 10.8 mg/m^3, respectively). Personal

full-shift (8-h TWA) monitoring data were not reported. Personal protective equipment was apparently not used. The urinary excretion of thiocyanates (in total volume of urine) did not exceed 9 mg/d. Cyanide workers were surveyed and reported symptoms (such as headache, weakness, changes in taste or smell, giddiness, irritation of throat, vomiting, dyspnoea and precordial pain). Although it was not stated whether these were concurrent with the present study or had occurred at some time in the past. Twenty workers had thyroid enlargement to a mild or moderate degree although there was no clinical manifestations of hyper or hypothyroidism. The cause of the goitre was assumed to be accumulating thiocyanate (thiocyanate inhibits iodine uptake by the thyroid). The authors measured ^{131}I uptake in the thyroid, and unexpectedly found a higher uptake rate in the exposed workers than in the unexposed controls. They explained this by two days of absence of exposure to cyanide and a corresponding decrease of thiocyanate in the serum, which might enhance the iodine uptake. Exposed workers also had significantly higher haemoglobin and cyano-methaemoglobin concentrations, and lymphocyte numbers in the blood as well as higher urinary thiocyanate levels than controls. These correlated with the cyanide concentrations in the air (El Ghawabi *et al*, 1975).

At first glance, the study of El Ghawabi *et al* indicates that occupational exposure to between 4.2 and 12.4 ppm cyanide is linked with development of goitre and a range of subjective symptoms. However, the Task Force believed that there are several inconsistencies in the reported data that suggest that this assertion is unreliable.

The authors suggested that there was a relation between the exposure to thiocyanate (metabolite of cyanide) and thyroid enlargement. However, the maximum excretion of the electroplating workers, reported by El Ghawabi *et al*, of 9 mg/d is not that far in excess of the 7 mg/d thiocyanate excretion of the average person in the French population without any cyanide exposure (Barrère *et al*, 2000). Indeed, from the toxicokinetics of thiocyanate according to Schulz (1984) it can be estimated that a daily excretion of 9 mg is equivalent to a thiocyanate concentration of 2 mg/l serum (29 µmol/l) (Table 63, Section 11.2.3), which is at the lower end of the background levels of thiocyanate in non-smokers (see below). It is therefore very unlikely that this level of thiocyanate could have caused the described effects on the thyroid, but would explain why there were no effects on thyroid hormones.

Secondly, urinary thiocyanate level is a reliable indicator of the level of cyanide absorbed and, indirectly, the levels that could have been present in the workplace. An excretion of 9 mg SCN^-/d is equivalent to an absorbed dose of 5 mg CN^-/d. If this 5 mg were present in the 10 m^3 of air inhaled during a working day and assuming there was no dermal absorption and 50% retention in the lungs, the average concentration in the workplace would have been about 1 mg/m^3 (0.9 ppm). This level of 0.9 ppm deviates strongly from the 15-minute breathing zone sample data reported by the authors (Section 5.2.2). Hence, if the reported hygiene data and clinical data are believed

to be reliable, then the urinary thiocyanate levels suggest that shift average (8-h TWA) exposure levels cannot be reliably extrapolated from the 15-minute breathing zone sample data presented.

The third inconsistency is that the rate of ^{131}I uptake in the thyroid was higher in cyanide exposed workers compared to controls. The authors explained this enhanced iodine uptake as being due to a reduced plasma thiocyanate level, as a consequence of from being away from work for 2 days. Whilst the concept of increased uptake by an up-regulated thyroid is plausible, this is unlikely to have been the sole reason since the half-life of thiocyanate in the body is approximately 3 days (Schulz, 1984). This means that the levels of plasma thiocyanate would have dropped by no more than 37% after 2 days, hardly sufficient to cause this effect. Either the withdrawal from exposure to cyanide must have occurred over a longer time period (i.e. past exposures must have been much higher) and/or the workers with goitre were also suffering from dietary iodine deficiency (as low dietary iodide in combination with an up-regulated thyroid could have led to a more pronounced increased ^{131}I uptake by the thyroid). Unfortunately, El Ghawabi *et al* did not provide information on the iodine excretion of the workers and therefore some concern must remain regarding the relevance of these findings.

The last inconsistency of the study by El Ghawabi *et al* is that the workers reported symptoms such as headache, weakness, vomiting, dyspnoea and precordial pain. However, these side-effects are typically associated with exposure to very high levels of cyanide, considerably higher than those reported by the authors. It is possible that these symptoms were caused by occasional peak exposure to high levels of cyanide, i.e. acute poisoning, and that these short exposures may not have been reflected by the hygiene data presented. If the worker questionnaire had not specified that only current symptoms should be reported, it is also possible that the above symptoms might be attributable to much higher (peak or TWA) levels in the past.

In summary, the presence of goitre in the exposed workforce was an indication of past exposure or iodine deficiency rather than the current situation, as goitre is not an acute development. No data were presented by the authors on historical exposure levels and the Task Force was unable to substantiate the assumption that past exposures were comparable to current levels. Subjective symptoms reported by the workers were based upon their occupational experience and were therefore unavoidably historical in nature. Comparable symptoms are typically reported in high level poisoning situations and may be indicative of high exposures in the past. The current clinical findings of a lack of an effect on thyroid hormones, despite the presence of goitre, are consistent with low urinary thiocyanate levels and relatively low cyanide exposure levels. Consequently, the link between development of goitre, subjective symptoms and the hygiene monitoring data presented by El Ghawabi *et al* cannot be substantiated.

Male workers (56 persons aged 27 to 51 [average 37.6] years) employed in copper electroplating and case hardening for 2 to 20 (average 11.5) years had also been exposed to HCN

(levels not given). The workers answered a questionnaire relating to a number of unspecific symptoms, such as headache, loss of appetite, chest pain and dizziness. The authors counted positive responses. Haematological investigations were made once in 34 workers and included haemoglobin, reticulocyte count, total and differential leukocyte count, and erythrocyte settlement time. Additionally, serum alkaline phosphatase levels, serum bilirubin and thymol turbidity were measured in 15 workers as an indication of liver function. Symptoms reported by the workers could not be related to exposure duration and the questions were too general to allow any firm conclusions on the influence of the work situation. In 19 workers, leukocyte counts were above normal. Erythrocyte settlement time was increased in 16 workers, while haemoglobin levels were lowered in 8 workers. (The Task Force noted that any relation to the workplace situation remained unclear.) Of the 15 workers that underwent blood clinical chemistry analysis, 10 showed elevated alkaline phosphatase levels and positive thymol tests while bilirubin levels were normal. (The parameters were relatively unspecific and did not allow any conclusions to be drawn on a possible work place related effect.) Additionally, the authors performed a number of behavioural tests on the exposed group and compared the performance with an age matched control group (age 23 to 48 y [average 34.8 y], sex not stated) of 34 persons of the same socio-economic group that did other work (not specified). The following tests were performed: Benton visual retention test (visual perception, visual memory, and visual constructive abilities), Koh's block test for visual ability and detection of visual spatial disturbances due to brain injury, digit symbol test for visual learning ability and motor speed (including coordination of eyes and hands) and mirror drawing test for detection of psychomotoric disturbances. (The authors did not report any details on the conditions under which the tests were performed nor the criteria for discrimination of effects versus normal performance. Also, the method of the statistical analysis was not disclosed.) The authors reported a significant difference between the exposed and control group in the loss of delayed memory, visual ability, visual learning, psychomotor activity ($p < 0.05$) and loss of immediate memory ($p < 0.01$). Thus, according to the authors, electroplating workers performance in the behavioural tests was significantly lower than that of controls (Kumar *et al*, 1992). The lack of details on the performance of the tests and the criteria for the reported results makes it impossible to evaluate the effects reported and their relevance. Although some of these effects may be attributed to CN exposure, the lack of exposure data prevents any firm conclusion about the dose-effect relationship.

Chandra *et al* (1980) measured concentrations of cyanide in air and thiocyanate in blood and urine in 23 electroplating workers in India, compared to 20 controls (matched for age, sex and socio-economic status) from the same company, who were never exposed to chemicals at the workplace. The workers were asked to avoid eating cyanogenic foods for at least 48 hours prior to collection of blood and urine samples. Smoking habits were also recorded. Venous blood was collected at the end of the work shift. Urine samples were taken before the work shift and subsequently at 2 hour intervals. After the end of the work shift, urine was collected until the start of the work on the following day. Personal air samples (20 min/h for 8 hours) were taken from

the breathing zone of the workers near the work baths and stationary area samples (20 min/h for 8 hours) at 8 points close to the floor. HCN levels close to the floor were reported to range between 0.2 to 0.8 mg/m^3 with an average of 0.45 mg/m^3 (0.18 - 0.71, 0.4 ppm), while in the breathing zone levels between 0.1 and 0.2 mg/m^3 (average 0.5 mg/m^3) (0.09 - 0.18, 0.45 ppm) were reported. The results on blood and urinary cyanide and thiocyanate levels reported by the authors are summarised in Table 58.

Table 58: Mean blood and urinary cyanide and thiocyanate levels in exposed and non-exposed workers (Chandra *et al*, 1980)

Group	Cyanide concentration (µg CN^-/100 ml)		Thiocyanate concentration (µg SCN^-/100 ml)	
	In blood	**In urine [a]**	**In blood**	**In urine**
Exposed non smokers (15)	18.3 (2 - 36)	5.4 (0 - 14.7)	0.42 (0.26 - 0.83)	0.57 (0.15 - 1.29)
Exposed smokers (8)	56 (23.2) [b] (10 - 220)	6.23 (0 - 18)	0.48 (0.16 - 0.92)	0.62 (0.15 - 1.65)
Control non-smokers (10)	3.2 (0 - 8.6)	2.1 (0 - 4.36)	0.04 (0.02 - 0.06)	0.08 (0 - 0.32)
Control smokers (10)	4.8 (0 - 9.4)	3.2 (0 - 8.45)	0.1 (0.07 - 0.18)	0.41 (0.24 - 1.2)

[a] 24-hour sample

[b] The value of 220 µg CN^-/100 ml was an outlier according to the authors. The value in brackets is the mean excluding this outlier

The exposed population had significantly elevated blood cyanide and thiocyanate levels and urinary thiocyanate excretion compared to the control population. The increase in in urinary cyanide excretion was not significant compared to controls. The authors mentioned only briefly that "the workers complained of typical symptoms of poisoning." No other indication of possible exposure related effects was given (Chandra *et al*, 1980).

Health surveys were made in 1980 and 1981 of workers exposed to cyanide fumes and aerosols in two Indian factories ('A' and 'B') where 22 and 18 persons worked in electroplating, and 18 and 53 in heat treatment, respectively. Data of the two plants were pooled for evaluation as workers had the same socio-economic status. Included were 30 control workers of factory B, but the control group was not characterised in the publication. Work place concentrations of cyanide were measured in the breathing zone of the workers near the cyanide baths and in the general environment (no details given). The total cyanide exposure per working day was given as total cyanide hours, calculated by the sum of the work place concentration measurements for different tasks multiplied by the respective times spent on these tasks (as indicated by the workers).

Clinical signs and symptoms reported by the workers were headache, heaviness, giddiness, lachrymation, itching and congestion of eyes, and coated tongue. The symptoms were ranked by the workers in grades of severity, although the authors stated that the clinical parameters were found inadequate for a diagnosis. The authors also reported on results of a behavioural investigation into delayed memory, visual ability, visual learning and, psychomotor ability. These were equally scored for severity. Blood and urinary levels of cyanide and thiocyanate were measured and the workers were told to avoid cyanogenic foods for at last 48 hours prior to sampling. Smokers and non-smokers were initially considered separately, but then put together again as the levels did not vary significantly between smokers and non-smokers. According to the authors, blood and urinary cyanide levels as well as blood thiocyanate levels did not differ significantly from controls (Chandra *et al*, 1988). However, the higher values of the ranges given, suggest that there may have been a number of high exposure situations (Table 59).

Table 59: Blood and urinary cyanide and thiocyanate levels in Indian electroplating workers
(Chandra *et al*, 1988)

Number of workers, factory	Cyanide concentration (µg CN^-/100 ml)		Thiocyanate concentration (µg SCN^-/100 ml)	
	In blood	In urine [a]	In blood	In urine [a]
40 exposed, A	0 - 4	0 - 17.5	0.07 - 0.9	0.1 - 8.1
71 exposed, B	0.54 - 28.4	0.27 - 29.4	0.05 - 2.8	0.22 - 5.23
30 controls, B	0 - 14	0 - 7.8	0.02 - 0.88	0.3 - 3.8

[a] 24-hour sample

The authors categorised the clinical and behavioural observations as well as the urinary thiocyanate measurements in three categories: ‘healthy’, ‘moderately diseased’ and ‘diseased’, and linked these to the cumulative exposure expressed as cyanide hours. There was a significant increase in moderately diseased workers from 2.13 to 2.51 cyanide hours (corresponding to 0.36 - 0.41 mg/m^3; 0.32 - 0.36 ppm) when divided by the exposure duration of 6 hours, as suggested by the authors. Interestingly, in the accompanying graphs all of the controls were characterised as diseased, but this was not further commented on (Chandra *et al*, 1988). Due to the findings in the controls, the lack of information on criteria for the classification of effects and on peak exposure situations, and the lack of details on the exposure measurements, the Task Force considered it impossible to draw any conclusions from this study on possible effects related to cyanide exposure at the workplace.

Thyroid activity was studied in 35 (non-smoking) copper-ply electroplaters that handled cyanide compounds in the cable industry. Levels of thiocyanate, TSH, T3 and T4 in serum of cyanide exposed workers who had been working in the job for more than 5 years were compared to 35 non-exposed controls (matched for age and dietary/smoking habits) from the same factory. None of the workers had a prior history of thyroid disease. Only one blood sample per person was taken (the sampling time was not stated in the paper). Serum thiocyanate concentrations were raised (316 µmol/l; $p < 0.01$) compared to control subjects (90.8 µmol/l), indicating exposure of these workers to cyanide at concentrations of approximately 9.2 mg/m^3 (calculated by the Task Force following Appendix H: cyanide exposure concentrations were not given in the publication.). Serum T4 (38.1 µg/l) and T3 (0.572 µg/l) concentrations were decreased ($p < 0.05$) and the TSH concentration increased (2.91 mU/l) ($p < 0.05$), compared to controls (T4: 60.9 µg/l, T3: 1.11 µg/l, TSH: 1.2 mU/l) (the Task Force normalised the concentrations per litre). The serum T4 level was found to be negatively correlated ($r = –0.363$, $p < 0.05$), whereas the TSH level was positively correlated ($r = 0.354$, $p < 0.05$) with the thiocyanate concentration in the exposed workers. T3 levels did not correlate significantly with serum thiocyanate concentrations. The authors suggested that the changes in thyroid hormone levels in the occupationally exposed group characterised by possible cyanide uptake via different routes of exposure (inhalation, dermal and oral) were indicative of an impaired thyroid function (Banerjee *et al*, 1997). The results have to be interpreted with caution as the external exposure of the subjects was not determined, only one blood sample per person was taken, and the sampling times were not given in the paper. Furthermore, the reported T3 and TSH values for the exposed individuals were within the normal range, as reported by the authors, and T4 levels were in the normal range, given by other sources (T4: 40.6 µg/l**,** TSH*:* 0.35 - 4.5 mU/l) (Ciba-Geigy, 1979; Zins and Trinh, 2003). Hence, the reported variations in thyroid hormone levels could be equally explained as being due to normal daily and/or inter-individual variations. Notwithstanding this, the thyroid effects are rather indicative of an adaptive rather than a pathological response.

Discussion and conclusions

The available literature, although limited, indicates that occupational exposure to cyanides in certain industries (silver reclamation and electroplating) is linked to thyroid enlargement (goitre) and a wide range of subjective symptoms. At first glance, the study of El Ghawabi *et al* (1975) suggests that these clinical effects are consistent with occupational exposures of between 4.2 and 12.4 ppm. The problem with accepting this interpretation is that the levels of urinary thiocyanate reported were too low to have caused the development of goitre (larger studies in humans refer: Cliff *et al*, 1986 in Section 9.2.2) and there were no effects on thyroid hormones. Indeed, the urinary thiocyanate levels reported were more consistent with levels of occupational exposure of 1 mg/m^3 (0.9 ppm, 8-h TWA). This brings into question the exposure data presented (36, 15-minute breathing zone samples) (Section 5.2.2). Because no shift average (8-h TWA)

monitoring data were collected it could be that these data, although perhaps taken during specific tasks, were not representative of the accumulative exposure across a full shift (unlike the urinary thiocyanate data). If current exposures were as low as the thiocyanate data indicates the question remains what caused the goitre and the subjective symptoms that were reported. Goitre is not an acute response and is therefore indicative of a more prolonged exposure to cyanide. Indeed, the absence of effects on thyroid hormones would suggest that the thyroid disturbance that caused the goitre had been some time prior to the current study. Similarly, the questionnaire is likely not to have distinguished between current and past symptoms so it is quite possible that past exposures might have been much higher and these had caused both the goitre and the subjective symptoms. Because of this uncertainty, it must be concluded that the study of El Ghawabi *et al* (1975) is not sufficiently reliable to be used as the basis for establishing an occupational NOAEL.

9.2.2 Non-occupational

Foods derived from cassava roots and leaves have been consumed on a regular basis by over 300×10^6 people throughout the tropics of Africa, Central and South America, Southern Asia, Micronesia and Melanesia (Cock, 1982). Variation in food preparation techniques has contributed to variable levels of residual dietary cyanide and resultant chronic cyanide intoxication in these regions. The published literature includes numerous epidemiological and cross-sectional studies on the health effects of dietary cyanide. These can be divided into those concerning thyroid function, the development of goitre and the importance of an adequate diet containing iodide and those concerning changes to the central and peripheral nervous system.

Thyroid effects and goitre

This review on goitrogenic effects is not intended to be exhaustive but rather has identified a small number of papers that are important to understanding the significance of the literature on occupational exposure to cyanides.

The prevalence of goitre in the Ubangi Mongala region of Zaire (present Democratic Republic of Congo) ranged from 27 to 60% in the male population and from 48 to 78% in the female population. The prevalence of cretinism ranged between 0.7 and 7.6%. The author stated that this severe endemic affected 1.5×10^6 inhabitants and constituted a major public health problem. It was suggested that administration of single injections of slowly resorbable iodised oil to the entire population could reduce goitre prevalence substantially, correct the iodine deficiency and restore normal thyroid function for a period of 3 to 7 years, depending on the parameter considered. A strategy of goitre and cretinism control in Central Africa, using iodised oil in an

attack phase and iodised salt in a follow-up phase, was proposed as part of other public health activities (Thilly *et al*, 1977).

Cliff *et al* (1986) studied thyroid function in a rural population in Mozambique that had been affected by an epidemic of spastic paraparesis reportedly attributed to dietary cyanide exposure from cassava. Levels of serum (250 µmol/l) and urinary thiocyanate (132 mmol/mol creatinine, corresponding to 1,137 µmol/l) were recorded for a sample of this population, indicating significant exposure to cyanide. Urinary excretion of iodine was within normal limits, indicating adequate dietary intake of iodine. The serum levels of the free T4 index were decreased and the serum free T3 index, T3/T4 ratio and TSH levels were raised. However, serum TSH values were all below 10 mU/l, indicating that no one had severe hypothyroidism. Only 3 subjects had enlarged goitre glands. This hormone pattern was suggestive of an adaptation to the antithyroid effect of thiocyanate, but not overt hypothyroidism (prevalence 1.1%). A follow-up study on school children also demonstrated high urinary thiocyanate levels, adequate intake of iodine, and absence of endemic goitre. The authors suggested that if iodine supply was adequate, the thyroid gland was capable of adaptation to a heavy body burden of thiocyanate without development of overt hypothyroidism or goitre. The authors mentioned that the serum thiocyanate levels in this study were higher than those reported by Bourdoux *et al* (1978), who studied serum thiocyanate levels in cassava-eating populations in the Ubangi region in Zaire (Democratic Republic of Congo). The prevalence of endemic goitre in this area was 60 to 70% of the population. Serum thiocyanate levels were 183 µmol/l and urinary thiocyanate concentrations 331 µmol/l. The population in Zaire suffered additionally from iodine deficiency as demonstrated by a low iodine excretion in urine.

Delange *et al* (1983) reported on a cassava-eating population in Zaire (Democratic Republic of Congo) with an endemic goitre rate of only 1.5% that was characterised by an adequate iodine intake. As concluded by Cliff *et al* (1986), the thyroid gland seems to be capable of adapting to relatively high thiocyanate levels if the iodine intake is adequate.

Three populations in Zaire (Democratic Republic of Congo) with different diets, all based mainly on cassava products, and different prevalence of goitre were studied to identify goitrogenic dietary circumstances. In Bas Zaire, goitre prevalence was 1.5%, no cretinism was observed and the prevalence of malnutrition in children (2 - 9 year old) was 16%. In Kivu, goitre prevalence was 12.5%, no cretinism was observed and the prevalence of malnutrition was 26%. In Ubangi, goitre prevalence was 76.8% with 4.3% cretinism and a malnutrition prevalence of 8.5% was determined. Urinary concentrations of thiocyanate, iodine and creatinine were determined in the different populations and compared to a matched control group in Brussels (Belgium). The urinary iodine-thiocyanate ratios (as µg I^-/mg SCN^-) were 8.8 in Brussels, 3.8 in Bas Zaire, 2.6 in Kivu and 1.9 in Ubangi. The authors concluded that dietary supply of iodine played a crucial role in the development of goitre in the presence of a cassava based diet. Protein malnutrition in

humans did not seem to critically impair the conversion of cyanide released from cassava to thiocyanate. The urinary iodine-thiocyanate ratio was a good indicator for the risk of endemic goitre. Endemic goitre was not found as long as this ratio was higher than 3 to 4 and was hyper-endemic when the ratio was below 2 (Hennart *et al*, 1982).

Jackson (1988b) studied the possible adaptation to serum thiocyanate overload associated with sublethal cyanide ingestion (in the form of cassava) in a group of 73 non-smoking indigenous Liberian adults. The mean daily intake of cyanide was determined to be 0.61 mg CN^-/kgbw (range 0 - 2 mg/kgbw). The mean serum thiocyanate levels were 22.1 µmol/l (range 0.5 - 208 µmol/l), but did not correlate with dietary cyanide intakes. The mean thiocyanate-sensitive antibody titre was 1,721 (range 10 - 2,560). Titres were positively correlated with dietary cyanide levels ($r = 0.22$, $p < 0.05$) and negatively correlated with serum thiocyanate levels ($r = 0.78$, $p < 0.01$). The authors suggested that in adults chronically ingesting 0.6 mg CN^-/kgbw and above per day, thiocyanate may be more rapidly and efficiently cleared from the serum, facilitated in part by immunological mechanisms.

Discussion and conclusion

Many millions of individuals eat processed cassava (containing cyanogenic glucosides) as part of their daily diet and this can result in the development of goitre. For over a quarter of a century it has been recognised that iodide plays a significant role in protecting the thyroid from thiocyanate toxicity and that maintenance of an adequate dietary iodide level can effectively eliminate this health risk. In the study of Jackson (1988b), it was shown that dietary intake of up to 2 mg CN^-/kgbw/d in humans did not result in any effect on the thyroid. (This is in direct contrast to the findings of the author's studies in miniature pigs published in the same year [Section 8.3]). The findings of Jackson (1988b) in humans are, however, in accord with the serum levels of thiocyanate (250 µmol/l) and lack of overt hypothyroidism reported by Cliff *et al* (1986) for a rural population in Mozambique with adequate iodide intake.

Clearly, adequacy of dietary iodide is a key parameter that has to be taken into consideration in the design, conduct and interpretation of studies with cyanides. Although this is not an extensive review, it is apparent that the NOAEL for goitrogenic effects in humans receiving adequate iodide is probably in excess of 2 mg CN^-/kgbw/d (the highest level found in the study of Jackson, 1988b).

Hennart *et al* (1982) reported that the development of goitre is critically dependent on the dietary balance between iodine and thiocyanate. Under normal conditions, the iodine-thiocyanate ratio is higher than 7 (µg I^-/mg SCN^-). The critical threshold for the development of goitre is a ratio of about 3 to 4. Hyper-endemic goitre and cretinism develop when the ratio is lower than 2. This is

in accordance with a study of Barrère *et al* (2000), who reported a relationship between thyroid volumes in French adults and the iodine-thiocyanate ratio, and indicated that thiocyanate exposure from different sources, such as smoking and diet may be critical in a population with borderline iodine intake. Thus a no-effect level for chronic cyanide exposure in humans will depend on the iodine status and cannot be set as an absolute figure. With an average urinary iodine level of 7 to 8 µg/100 ml (0.07 - 0.08 µg/ml) in low iodine regions of Europe (Barrère *et al*, 2000), the urinary thiocyanate levels should not exceed 20 µg SCN^-/ml. Taking into account a typical background thiocyanate urine level of about 6 to 7 µg/ml, as measured by Barrère *et al* (2002), this would allow for an additional (exogenous) exposure to cyanide equivalent toa maximum of 13 µg SCN^-/ml (as measured in the urine) without any adverse effect on the thyroid.

Neurotoxicity due to exposure to cyanides

The central nervous system is the primary target for cyanide toxicity in humans and animals. Short-term (single) inhalation of high concentrations of cyanide provokes a brief stimulation of the central nervous system followed by depression, convulsions, coma and death in humans (Potter, 1950; Peden *et al*, 1986).

The severity of neurological effects in humans after acute oral exposure to cyanide is dose-related. The symptoms vary from tremor and headache (Chen and Rose, 1952; Lasch and El Shawa, 1981) to deep coma and death following central respiratory arrest (Lasch and El Shawa, 1981; Thomas and Brooks, 1970). Pathological changes that may occur in the central nervous system during acute exposure to high doses may complicate recovery.

Convulsions and coma were also reported in humans following acute dermal exposure to cyanide (Dodds and McKnight, 1985; Trapp, 1970).

Ballantyne (1987b) described some severe intoxications with cyanide, in which permanent brain damage with concomitant personality change was found after recovery.

Parkinson-like syndrome

Progressive Parkinsonism, dystonia and apraxia of eye opening were seen after cyanide poisoning. Computed tomography and magnetic resonance imaging showed lesions in the basal ganglia cerebellum and cerebral cortex consistent with reported pathological findings (Carella *et al*, 1988). The authors described the case of a 46 year old woman and her husband, who were brought to the hospital after drinking from a bottle of a soft drink contaminated with

cyanide. The man died before arrival at hospital. On admission the woman was found to be in light coma. An analysis of gastric aspirate confirmed the presence of cyanide. Computed tomography and cerebrospinal fluid examination were normal. The patient showed occasional decerebrate posturing but slowly recovered in 2 weeks. One year later the patient went to hospital with impairment of spatial and visual memory. Five years after, she showed Parkinson features with abnormal posture of the mouth and tongue and disorder of eye opening. There was clinical and radiological evidence of striatal involvement.

Severe Parkinson syndrome was described in an adolescent boy after a suicide attempt. The subject drank alcohol and had a preoccupation with synthetic drugs such as lysergic acid diethylamide (LSD), sleeping pills (amobarbital sodium, secobarbital sodium, pentobarbital sodium) and a variety of 'wake up pills'. He had swallowed amobarbital sodium and KCN tablets and became comatose, not responding to painful stimuli. After resuscitation, he regained consciousness in 7 hours. Neurological examination 4 month later revealed marked generalised rigidity and bradykinesia. A diagnosis of Parkinson was made. Autopsy after his death showed that the pathological disorder affected mainly the globus pallidus and putamen. The authors suggested that Parkinson may have resulted from anoxia after his cyanide intoxication (Uitti *et al*, 1985). Given the exposure to a number of other synthetic drugs, no firm conclusions can be drawn on the causal relationship of the observations with cyanide ingestion.

A 16 year old man had ingested 1 g KCN in 1969. On admission to hospital he was comatose, his blood pressure was undetectable and he had a deep respiratory depression with a heart rate of 70 beats/min. The patient received cardio-pulmonary resuscitation and sodium thiosulphate *i.v.* and regained consciousness after 3 hours. After 24 hours he was, according to the authors, "neurologically intact" (no details on examinations given) and the results of a general physical examination were normal. A few days later he developed slurred speech, involuntarily altered movements of the right hand and a generalised dystonia with hyperextension of his lower limbs and trunk. He was unreactive to chlorpromazine treatment. The symptoms were aggravated after 29 days and included inability to walk or speak, hypertonic facial muscles, involuntary contraction of limb muscles when he attempted to walk. His mental state was normal. He had a positive response to an apomorphine test and showed improvement with levodopa (an anti-Parkinson drug) treatment (3,600 mg/d). After 38 days on levodopa, his condition had improved and subsequently improved further without treatment. A follow up after 21 years showed only minimal neurological sequelae such as mild dystonia and athetoid involuntary movements of both hands. Computed tomography showed bilateral putaminal hypodensities and a mild cortical atrophy. Audiologically and visually evoked potentials in the brain stem were normal and did not suggest an effect. The authors concluded that the Parkinson-like syndrome developing as a delayed reaction to acute cyanide poisoning was a largely reversible effect that has no long-term effect on the brain stem (Valenzuela *et al*, 1992).

Rosenberg *et al* (1989) described a case of a 46 year old man who ingested 1,500 mg KCN in a suicide attempt. Within 15 minutes he became unresponsive, had shallow respiration and cyanosis of his hands and feet. The systolic blood pressure was 90 mm. On admission to hospital, the serum cyanide level was 3.8 μg/ml (normal < 0.5 μg/ml). The patient was treated 25 minutes after ingestion of cyanide by *i.v.* injection of sodium thiosulphate and sodium nitrate, and transferred to a hyperbaric oxygen chamber. He regained consciousness within 2 hours and a neurological examination after 3 days was normal except for a slight difficulty with tandem gait and halting speech. After 2 weeks he developed progressive slowness of gait, masked facies and hypophonia. Neurological examination 3 weeks after the suicide attempt revealed a severe Parkinson-like syndrome with marked bradykinesia, masked facies, slow, shuffling gait, mild rigidity and a weak, dysphonic voice. Computed tomography and magnetic resonance imaging 6 months later revealed no abnormal findings. Detailed neurofunctional testing, using 'Minnesota multiphasic personality inventory scores' and higher level function tests, revealed impaired motor function, but no other abnormalities. Treatment with levodopa/carpidopa did not significantly improve the symptoms and was discontinued after 10 months. Repeat magnetic resonance imaging after 12 months revealed multiple bilaterally symmetric areas of low signal intensity in the globus pallidus and posterior putamen with T1 imaging that became high signal with T2 imaging. Distribution of dopamine in the brain was studied using 6-fluorodopa and positron emission tomography and revealed the same decreased activity in the basal ganglia and marked decreased activity in the posterior basal ganglia that is observed in Parkinson patients. The authors concluded that their findings were either indicative of a specific affinity of cyanide to the brain regions of globus pallidus and putamen, or of a secondary effect of the cerebral hypoxia resulting from cyanide intoxication.

A 39 year old man had swallowed an unknown amount of NaCN in an attempt to commit suicide. When he was found, he was unconscious and had respiratory arrest. The patient was treated with assisted ventilation and peritoneal dialysis and underwent abdominal surgery because of partial gastric necrosis. He regained consciousness several days later, but was apathetic, showed loss of facial expression and reduced speech ability. When walking he had balance problems. Immediate computed tomography did not reveal any abnormalities, but a computed tomography one month later showed bilateral basal ganglia and left frontal deficiencies. About one year after the attempted suicide, relevant clinical findings such as masked facies, greasy skin, axial and limb rigidity, generalised bradykinesia, resting tremor in the arms, gait impairment and unstable posture were reported. His speaking ability was seriously impaired, but mental ability and understanding was normal. Two years later, the subject was anarthic (unable to speak) and confined to a wheel-chair. Other symptoms included generalised hyper-reflexia and permanent flexed posture of the right elbow. Computed tomography of the brain at that time showed mild cortical and subcortical atrophy and bilateral lentiform lutencies in the putamen and external lobus pallidus. Various medications with anti-Parkinson drugs (levodopa, bromocriptine) and psychopharmaca (e.g. diazepam, imipramine) were without any clinical improvement. Acute

challenge with apomorphine did not produce any effect on the motor function (Grandas *et al*, 1989).

A 28-year old man had ingested 800 mg of KCN in a suicide attempt. He was unconscious when he was admitted to hospital, and had shallow respiration and dilated pupils. The blood thiocyanate level was 23 mg/l and the alcohol level 1,090 mg/l. The patient was injected *i.v.* with sodium thiosulphate, sodium nitrate, dopamine and sodium bicarbonate and received hyperbaric oxygen. After regaining consciousness, the patient was lethargic, did not speak, frequently vomited and had low-grade fever. The subject had depression and a history of alcohol abuse for several years. It was found out that he had already attempted suicide with a low amount of cyanide 3 months before but had recovered without treatment. Over the next 3 weeks he developed extra pyramidal signs (Parkinsonian syndrome). Brain magnetic resonance imaging after 3 months revealed symmetric abnormalities in the basal ganglia, in particular in the globus pallidi, which remained unchanged after 15 months, when additional mild cerebellar atrophy was also diagnosed (Feldman and Feldman, 1990).

Conclusion

A few cases have been published that relate cyanide exposure to Parkinson-like symptoms. They all refer to an acute over-dose of cyanide, mostly in connection with attempted suicide, that were treated in a comatose stage. The symptoms seem to be related to brain damage of the basal ganglia, the globus pallidus and putamen that appear to be the most sensitive brain regions with regard to cyanide toxicity. There is no indication from the available data that repeated low dose exposure to cyanide could have similar effects.

Neurotoxicity due to ingestion of cassava

The population of 21 villages in the Bandundu region of Zaire (Democratic Republic of Congo) was screened for signs of konzo, a symmetric spastic paraparesis characterised additionally by disturbances of vision and speech. Additionally, the size of the thyroid gland was examined and a few other symptoms including spinal abnormality, oedema and ulcers of the lower limbs, ankle clonus and reflexes. Individuals exhibiting the symptoms were questioned about their diet in particular at the time of the onset of symptoms. Clinical records of the individuals were also reviewed. Of 6,764 inhabitants, 110 persons revealed symptoms of konzo (prevalence 16/1,000), 11 people had slightly enlarged thyroid glands, of which only 2 were visible. The subjects of the study reported that the onset occurred within 1 to 3 days, mostly during the dry season. In some individuals, exacerbations occurred during the same season. The authors discovered that this often coincided with periods in which cassava, grown as the main staple food, was insufficiently

processed, causing acute toxicity symptoms such as dizziness, fatigue, headache, vomiting and diarrhoea. The subjects stated that they had, almost exclusively, eaten cassava and no protein during the period before the onset of the disease. Many also had experienced repeated acute intoxications (Tylleskär *et al*, 1991).

Osuntokun (1968, 1981) and Osuntokun *et al* (1969) studied cases of tropical neuropathy in Nigeria that were characterised, *inter alia*, by paraesthesia and dysaesthesia in the distal parts of the limbs, vision disturbances, impaired hearing and signs of peripheral neuropathy with ataxia of gait.

Osutokun (1968) studied 84 patients (43 males and 41 females) exhibiting the above symptoms and compared them to a number of control humans not suffering from the same symptoms. Plasma and urinary thiocyanate, urinary water-soluble vitamins, haemoglobin, cholesterol and vitamin B_{12} in blood were determined in the patients. All patients had a history of a diet in which the staple food was cassava. An alcoholic beverage containing a high amount of methanol was also reported to be common in the area, but the authors could obtain no history of alcoholism. The majority (60%) of patients had had symptoms for more than 3 years. The author reported no difference in haemoglobin levels, serum albumin, globulin or total protein levels between patients and controls from the same region. Serum cholesterol as an indicator of calorie consumption was lower in patients than in controls. Low urinary riboflavin and thiamine levels were found in patients and controls, serum foliate and vitamin B_{12} levels were relatively high in both groups. Plasma and urinary thiocyanate levels were raised in patients with the ataxic syndrome. Glucose tolerance tests, liver function tests and thyroid function were normal in most of the patients.

Osuntokun *et al* (1969) reported a study in two Nigerian villages that were located in high cassava consuming and low cassava consuming areas, respectively. The populations were otherwise comparable. In the first village, cassava consumption was 64% and in the second 10% of the diet. Alcohol and tobacco consumption were higher in the first community compared to the second. Average serum thiocyanate levels of 30 people in the first village were reported to be 6.7 µmol/l, in the second (n = 43) 4.6 µmol/l. Mean serum vitamin B_{12} was lower in the first group than in the second. The incidence of tropic neuropathy was 23% in the first and 1% in the second community.

Osuntokun (1981) reviewed a number of studies related to the origin of tropic neuropathy in Nigeria. He reported that plasma and urine levels of thiocyanate were significantly higher in patients than in controls. The values for plasma (µmol/100 ml) were, respectively, 11.3 (n = 364) and 2.4 (n = 206), and for urine (µmol/kgbw/24 h) 2.4 (n = 136) and 0.6 (n = 40). Sulphur-containing amino acids in urine were undetectable in 60% of the patients and greatly reduced in other patients. The free cyanide level in plasma (not plasma bound) was higher in patients (0.051 µmol/100 ml [n = 37]) than in controls (0.031 µmol/100 ml [n = 40]). The total

plasma cyanide concentration in patiens was also elevated (0.1 µmol/100 ml [n = 177] compared to controls (0.03 µmol/100 ml [n = 106]). The endemic foci of the disease were closely related to areas with a high level of cassava consumption. The author stated that the daily intake of cyanide in the endemic areas may be as high as 50 mg, which is close to a reported lethal dose of 60 mg for man.

Similar results were reported by Banea-Mayambu *et al* (1997) in the Bandundu region of Zaire (Democratic Republic of Congo). In the dry season, the urinary thiocyanate concentration was higher in konzo-affected villages than in those that reported no konzo cases. Urinary inorganic sulphate was generally very low. The authors found a better correlation of konzo with urinary concentrations of linamarin (intact glucoside) than thiocyanate, indicating that the intact glucoside may have contributed to the disease.

Cliff and Nicala (1997) described 9 cases of konzo, which occurred during an agricultural crisis in a cassava-growing area in Mozambique. These patients had acutely developed the syndrome and were re-examined 10 to 14 years later. The konzo outbreaks were initially associated with eating insufficiently processed bitter cassava and a low intake of essential amino acids. In most of the patients the symptoms persisted over the years. Functional improvement was reported in 4 patients.

Cliff *et al* (1997) reported an epidemic of konzo in Mozambique associated with cassava consumption and cyanide exposure. Owing to war, communities had turned to bitter cassava and took short cuts in its processing. The epidemic lasted 2 years. There were 384 patients treated in rehabilitation centres. The prevalence rate in a badly affected area was 30/1,000. School children (77 urine samples) had high mean levels of urinary thiocyanate (207 µmol/l) and linamarin (80 µmol/l), and low levels of inorganic sulphur (4,877 µmol/l), indicating an increased intake of insufficiently processed cyanogenic glucosides and a low intake of protein-rich foods.

In another study by the same authors, 397 school children were examined for ankle clonus in three districts in Mozambique affected by konzo. They analysed 131 urine specimens for urinary thiocyanate, linamarin and inorganic sulphate. The geometric mean concentrations were 163 and 60 and 4,400 µmol/l, respectively. The proportion of children with clonus varied from 4 to 22% between sites. Children with ankle clonus had significantly higher urinary thiocyanate concentrations than those without clonus. Sulphur and linamarin concentrations were not significantly different. The low sulphur concentration in urine reflected sulphur amino acid deficiency in all districts (Cliff *et al*, 1999).

In the three papers of Cliff *et al*, no indication is given of the amount of cassava ingested by the examined population, so no quantitative correlations of the possible intake of cyanide and the observed effects can be derived.

Summary and evaluation

In all studies of cassava related neurological diseases, the outbreaks seem to be related to a high intake of insufficiently processed cassava with high levels of cyanide in combination with a low protein diet and a lack of essential sulphur-containing amino acids. As indicated in the studies of Osuntokun (1968) and Osuntokun *et al* (1969), the disease might be related to increases of free plasma cyanide concentrations under the above-mentioned dietary conditions. In the cases where cyanide intake was estimated, it was close to lethal concentrations and associated with acute symptoms that were probably survived because of the retarded liberation of cyanide from the cyanogenic glucosides. Transient high acute cyanide concentrations could have led to damage of brain cells. However, as indicated by Banea-Mayambu *et al* (1997) and Cliff *et al* (1997), the free glucosides themselves (e.g. linamarin) may also be causally related to the syndrome. Furthermore, other ingredients of cassava have been detected that could also be involved in the development of neurogenic disorders (Section 8.3.1). It can be concluded that neurological diseases related to diets consisting of cassava with high cyanogenic glucoside content are multifactorial diseases that can only partly be attributed to what seem to be high cyanide intakes.

10. TOXICITY OF THIOCYANATE AFTER REPEATED EXPOSURE AND ITS RELEVANCE FOR HUMAN EXPOSURE

The repeated-dose toxicity of cyanides has been related to the toxicity of the detoxification product thiocyanate. Therefore the toxicity of thiocyanate will be discussed briefly in this chapter. The Task Force has concentrated on a number of reviews and key papers on thiocyanate toxicity, and has not performed an exhaustive review of the literature.

10.1 Animal studies

In dogs, repeated oral doses of 100 mg SCN^-/kgbw (duration of study not stated) caused progressive body weight loss, apathy, ataxia and deaths. In a 3-month study in dogs, microcytic anaemia, decreases in blood cholesterol and serum protein levels as well as lesions in bone marrow and spleen and an inhibition of the iodine uptake in the thyroid were reported. Dogs receiving oral doses of 100 to 200 mg KSCN/kgbw for 63 to 96 days developed anaemia and showed a decrease in eosinophilic neutrophils. Fatty infiltration and necrosis were observed in the liver and epithelial swelling in the kidney. Furthermore, the dogs developed goitre (several studies reviewed by Weuffen, 1982).

De Jong and de Wied (1966) studied the effects of potassium thiocyanate (0, 0.3, 0.9% KSCN in drinking water) on thyroid function and blood pressure of female Wistar rats (130 - 170 gbw) for 5 or 16 days. Controls received tap water. The diet was enriched with iodine (0.5 to 0.8 mg/kg diet). (The respective doses can be calculated as 0, 400 or 1,200 mg KSCN/kgbw/d or 0, 239 or 718 mg SCN^-/kgbw, based on an estimated drinking water consumption of about 20 ml/d for young adult female rats and the average reported body weight of 150 g from the study). Thyroid activity was determined by measuring the uptake of radiolabelled iodine (^{131}I) and the subsequent release of thyroidal ^{131}I, by counting the radioactivity in the neck region under light ether anaesthesia. Administration of 0.9% KSCN in drinking water for 14 days resulted in a significantly increased thyroid weight. Administration of 0.3 and 0.9% KSCN in drinking water for 5 days significantly reduced the ^{131}I uptake in a dose-related manner and the release of thyroidal radio-iodine was increased. No increased release in thyroidal ^{131}I was observed in hypophysectomised rats or rats with a lesion in the hypothalamus that depressed pituitary TSH release. This indicated that the goitrogenic action of KSCN was dependent on the increase of pituitary TSH. The hypotensive effects were independent of the effects on the thyroid. A NOAEL was not established in this study.

In a 2-year study, F344 rats (20/sex/group) were given sodium thiocyanate (0.32% NaSCN) in drinking water for 5 days per week for up to 112 weeks. The dose levels were approximately 250 mg NaSCN/kgbw/d as stated by the authors (corresponding to 179 mg SCN^-/kgbw/d).

No increase in mortality and no increase in tumour incidence were reported compared to controls. No other effects were reported in the publication (Lijinsky and Kovatch, 1989).

10.2 Pharmacokinetic information

Distribution and elimination kinetics seem to be similar in different species. In rabbit, guinea pig and rat 80 to 95% of orally administered thiocyanate is excreted in the urine within 5 to 14 days. Blood thiocyanate levels in rats fed diets with different thiocyanate content were not much affected by the dose. Renal clearance [a] increased in proportion to the quantity of thiocyanate that was administered. In rats fed on a low iodine diet with restricted feeding (6 h/d), clearance of 0.1 mg SCN^-/d was 0.42 ml/min and rose to 1.5 ml/min when the supplement was 5 mg SCN^-/d. In dogs, clearances of 0.1 to 7.3 ml/min were reported when the plasma concentration was 10 mg SCN^-/100 ml (100 mg/l) (Wood, 1975).

The kinetics of radiolabelled thiocyanate ($^{35}SCN^-$) were studied in male Sprague-Dawley rats (340 - 360 gbw). Five rats received *i.p.* injections (1 ml) of 50 µg $^{35}SCN^-$ (1.8 µCi) as tracer, while a further 4 rats received the same amount of tracer in addition to 5 mg of non-radiolabelled SCN^- (as NH_4SCN). The plasma level resulting from the latter was approximately 5 mg SCN^-/100 ml. Blood samples were analysed at 1, 2, 4, 6 and 24 hours after injection. While the half-life of thiocyanate was 4.42 days in rats receiving the tracer dose only, it was 0.31 days in rats that received additional thiocyanate, i.e. elimination was faster in the latter. In normal rats, the background concentration of thiocyanate was 0.5 to 0.6 mg SCN^-/100 ml (5 - 6 mg/l) of serum and, according to the authors, this had a plasma half-life of 2 to 4 days. The experiment indicated that thiocyanate excretion was dependent on the thiocyanate blood levels and could be increased at higher levels (Funderburk and Middlesworth, 1971).

In healthy humans receiving single doses of 1.2 to 1.5 g NaSCN, 96 to 99% of the dose was excreted in urine within 5 to 14 days; 3 to 7 mg/d were excreted in faeces. After prolonged exposure to thiocyanate, urinary excretion was increased and serum levels decreased (Weuffen, 1982).

Pullman and McClure (1954) studied the excretion and re-absorption of thiocyanate in 6 male volunteers (age 30 y) with different levels of chloride excretion and diuresis, in relation to different blood levels of thiocyanate (injected as 5% NaSCN solution). Data were obtained for 122 clearance periods with urine flows ranging from 1 to 23.2 ml/min per 1.73 m^2 body surface. Thiocyanate serum concentrations varied from 0.25 to 4.22 mmol/l serum (14.5 to 24.5 mg/l). The re-absorption rate of thiocyanate was always linearly related to the serum concentration and

[a] Volume of plasma from which a substance is completely removed by the kidney in a given amount of time (min)

paralleled the water re-absorption. The elimination half-life in humans via urinary excretion was determined to be about 3 days (range 1-5 d) in healthy human volunteers and 7 to 9 days in patients with impaired renal function. The renal thiocyanate clearance was between 4.32 and 5.65 ml/min. Extra-renal elimination accounted for about 15% of the thiocyanate removal. Therefore, according to the authors, the elimination half-life of thiocyanate could not be longer than 15 to 20 days, even in anuric patients (Schulz *et al*, 1979).

Schulz *et al* (1978) calculated steady-state concentrations of thiocyanate after *i.v.* administration of sodium nitroprusside [a]. It was assumed that the cyanide liberated from sodium nitroprusside was quantitatively converted to thiocyanate. With a distribution volume for thiocyanate of 15 l for a 60 kgbw person (0.25 l/kgbw), as determined by the authors in previous clinical studies, and an elimination half-life of 7 days in a person with somewhat impaired renal function, they calculated a steady-state concentration of 85 mg SCN^-/l that would be reached after 4 weeks of treatment with 125 mg sodium nitroprusside per day. This fitted well with thiocyanate levels in clinical cases reported by the authors. The dose would correspond to a tolerable long-term *i.v.* dose of about 55 mg CN^-/d for a 60 kgbw individual or 0.92 mg/kgbw/d.

10.3 Human experience

In humans, physiological levels of thiocyanate in serum ranged from 4.4 to 5.5 mg/l for non-smokers and from 2.8 to 9.3 mg/l for smokers. Urinary excretion amounted to 2.1 to 12 mg/l in non-smokers and 3 to 17.5 mg/l in smokers. Levels may vary considerably with the diet. High levels of thiocyanate are, for example, found in cabbage and mustard (Weuffen, 1982; Aubry *et al*, 1988).

Information on possible adverse effects of thiocyanate in humans can be derived from human experience with the former use of sodium or potassium thiocyanate as an antihypertensive drug and experiences with antihypertensive therapy with sodium nitroprusside, which liberates 5 mol CN^-/mol of sodium nitroprusside. Several authors have established that in humans, dose levels of 125 mg SCN^-/d are usually well tolerated in patients with normal renal function, while 250 mg SCN^-/d could cause adverse side effects after prolonged exposure (Weuffen, 1982; Schulz *et al*, 1978; Schulz, 1984). At therapeutic dose levels, the tolerable blood levels ranged from 50 to 120 mg SCN^-/l serum. Anaemia was observed at blood levels of 100 to 200 mg/l serum, and visual and hearing disturbances at 150 mg/l serum (Weuffen, 1982). A decrease in T3 and T4 serum levels and in increase in the occurrence of goitre were observed after prolonged treatment leading to blood serum levels of 200 mg SCN^-/l (Schulz *et al*, 1978). Barker (1936) and Barker *et al* (1941) described their experience with thiocyanate therapy for hypertension of 45

[a] $Na_2[Fe(CN)_5NO] \times 2H_2O$, molecular mass: 298

and 246 patients that received thiocyanate therapy for 1 to 4, or 4 to 10 years, respectively. Oral doses of 300 mg/d usually led to blood levels of 50 to 100 mg SCN^-/l, but in some cases higher or lower levels were obtained. When blood levels were maintained between 80 and 120 mg/l, most of the side effects could be avoided. In the first group, 3 patients and in the second group, 20 patients were identified who had indications of hypothyroidism. In the publications, the corresponding blood thiocyanate levels were not given. The renal function and the iodine status of the patients were not controlled. The authors reported that a reduction of the thiocyanate dose to 65 to 130 mg SCN^-/d led to a recovery within 2 weeks. This would be suggestive of a human NOEL of about 20 to 40 mg SCN^-/l blood.

Dahlberg *et al* (1984) studied a possible effect of thiocyanate added to milk (as a preservative against bacterial contamination) on thyroid function in 37 human volunteers (28 females, 9 males) aged between 16 and 54 years. The experiment lasted 12 weeks. All persons were reported to have normal health status, no history of thyroid function disturbance and "normal dietary habits" including a normal iodine status. Five persons were smokers (5 to 20 cigarettes/d). The individuals drank (2 ×/d) 200 ml of milk supplemented with 20 mg/l of thiocyanate, resulting in an additional uptake of 8 mg SCN^-/d. Venous blood and urine samples were collected before the start of the experiment and after 4, 8 and 12 weeks. An examination of the thyroid was also performed. Blood samples were analysed for haemoglobin, haematocrit, thrombocytes, leucocytes, creatinine, T4, T3, thyrotropic hormones, and serum thiocyanate. Urinary thiocyanate and iodine levels were also determined. No adverse symptoms were observed during the study and all clinical chemistry parameters, except the thiocyanate levels, remained unchanged. Thiocyanate serum levels of the non-smokers increased from 4 to 7.8 mg SCN^-/l after 4 weeks and decreased again to 6.9 and 7 mg/l after 8 and 12 weeks. The increases were smaller in the smokers. Urinary excretion of non-smokers paralleled the serum concentrations for 4 weeks when it increased from 16 mg SCN^-/g creatinine to 20 mg/g creatinine; excretion decreased to 17 mg/g creatinine in week 12. In smokers, no significant changes compared to pre-exposure levels were observed. Urinary iodine concentrations (200 µg/g creatinine) did not change significantly during the experiment. The daily average urinary excretion of creatinine was about 1 g for females and 1.4 g for males. Thus an increased oral uptake of 8 mg SCN^-/d for 3 months did not have any adverse effect on the thyroid in healthy human subjects in this study.

Dahlberg *et al* (1985) investigated the effect of thiocyanate-supplemented milk on iodine deficient subjects in the Dafur region of Western Sudan, an area with a high rate of endemic goitre due to dietary iodine deficiency. Thirty-one males and 24 females (13 - 17 years old) who all had goitres in stage I and II, but which were clinically euthyroid, took part in the study. They were divided into 3 groups with equal sex and age distribution and were drinking (250 ml/d) milk (100 µg I^-/l) for 4 weeks, containing 3.6 mg SCN^-/l (1 group) or 19 mg SCN^-/l (2 groups). The daily additional thiocyanate uptake was 0.9 or 4.75 mg respectively. Before the start of the trial, one of the 19 mg SCN^-/l groups received iodinated oil (400 mg I_2/person). Venous blood and

urine, sampled before the start of the study and after 4 weeks, were analysed for creatinine, thiocyanate, iodine, serum T4, T3 and TSH, including free T3 and T4 indices. Reference ranges were 1.2 to 2.8 nmol/l for T3 and 67 to 153 nmol/l for T4. TSH levels were below 8 mU/l. The free T3 index was 1.2 to 2.8 and the free T4 index 67 to 153. The results with regard to thyroid hormone levels, TSH, thiocyanate and iodine are summarised in Table 60. Serum creatinine levels were normal in all subjects before and after the trial.

Table 60: Thyroid hormone levels, TSH, thiocyanate and iodine (Dahlberg *et al*, 1985)

Group (mg SCN^-/l milk) / Duration	**Serum level**				**Serum index**		**Urine level**
	SCN^- (mg/l)	**TSH (mU/l)**	**T3 (nmol/l)**	**T4 (nmol/l)**	**Free T3**	**Free T4**	**I^- (µg/g creatinine)**
3.6							
0 wk	1.8 ± 0.2	2.9 ± 0.2	2.4 ± 0.1	115 ± 5	1.9 ± 0.1	93 ± 3	48.8 ± 4.7
4 wk	1.8 ± 0.1	2.5 ± 0.2 [a]	2.1 ± 0.1 [b]	100 ± 6 [b]	1.9 ± 0.1	90 ± 3	69.2 ± 7.8 [a]
19							
0 wk	1.7 ± 0.1	2.4 ± 0.3	2.0 ± 0.1	111 ± 5	1.6 ± 0.1	87 ± 3	42.2 ± 4.7
4 wk	3.5 ± 0.1 [a]	2.0 ± 0.2 [a]	1.7 ± 0.1 [a]	99 ± 4 [b]	1.5 ± 0.1	86 ± 3	52.1 ± 5.5 [b]
19 + iodine							
0 wk	1.8 ± 0.2	2.4 ± 0.2	1.7 ± 0.1	105 ± 4	1.4 ± 0.1	84 ± 2	41.8 ± 3.5
4 wk	3.4 ± 0.5 [b]	1.8 ± 0.1 [b]	1.6 ± 0.1	98 ± 3	1.5 ± 0.1	83 ± 3	138.1 ± 15.8 [c]

[a] Significant $p < 0.001$
[b] Significant $p < 0.01$
[c] Significant $p < 0.05$

Serum thiocyanate levels did not increase significantly in the group receiving 3.6 mg SCN^-/l milk, but in the two other groups a significant increase was observed. Serum levels of TSH, T3 and T4 were in the normal range for all groups, both at the beginning and the end of the study. TSH levels had significantly decreased after 4 weeks in all groups, the values of the free T3 and free T4 indices did not change. Urinary iodine levels increased in all groups, more pronounced in the group receiving additional iodine.

The authors attributed the increased excretion of iodine in all treatment groups to the iodine content of the milk. The increased thiocyanate concentration did not seem to influence the level of iodine excretion. The increases in T4 and T3, but not free T4 and T3 indices, indicated – according to the authors – an effect on binding proteins rather than on hormone production that was explained by the additional protein intake through the milk. The decrease in TSH levels in all groups was interpreted as an effect of the additional iodine intake through the milk. The intake of milk with a thiocyanate level of 19 mg/l in the presence of iodine (100 µg/l) did not have an apparent negative effect on the thyroid function in an iodine deficient population (Dahlberg *et al*, 1985).

A worker who handled cyanide in an open hot bath complained of repeated acute symptoms of nervousness, giddiness, headaches, loss of appetite, nausea and vomiting during work. When he was away from work the symptoms disappeared. The patient did not show an impairment of thyroid function. The author treated the individual with daily doses of sodium thiocyanate (1.4 g NaSCN) in order to find out if the symptoms were related to thiocyanate. Thiocyanate blood levels were recorded every day during the treatment. From serum levels of 19 mg SCN^-/100 ml (190 mg/l), the patient reported the same symptoms as at the work place. The symptoms disappeared immediately after cessation of treatment and a fall in thiocyanate blood levels below 19 mg/100 ml (Wüthrich, 1954).

The uptake of radiolabelled iodine (^{131}I) was measured in 10 individuals with non-toxic goitre and base serum levels of 5 µg SCN^-/ml after *i.v.* injections of 250 (n = 6) or 500 mg (n = 4) thiocyanate. The initial uptake rates were in the normal range. After injection of thiocyanate, the ^{131}I uptake was inhibited by 14% at 250 mg group (serum levels 14 µg SCN^-/ml), and by 34% at 500 mg (23.5 µg SCN^-/ml serum) (Bourdoux *et al*, 1978).

Conclusion

From the available data it can be concluded that the kinetics of thiocyanate are similar in humans and experimental animals. Urinary excretion is the main route of elimination and it increases with increasing plasma levels of thiocyanate. In humans, the distribution volume for thiocyanate was 0.25 l/kgbw and the elimination half-life 2.7 days in healthy individuals or up to 9 days in

subjects with impaired renal function. Human experience suggests that serum levels of 20 to 40 µg SCN^-/ml would not lead to adverse effects in healthy humans. However, in individuals with pre-existing goitre due to iodine deficiency, such levels could lead to an aggravation of the disease. Similarly, individuals with impaired renal function would tolerate lower levels of serum thiocyanate due to the slower excretion.

11. HAZARD ASSESSMENT

11.1 Environmental hazard

11.1.1 Environmental fate

Atmospheric fate

Due to the relatively low pKa value of HCN (> 9) and its relatively high vapour pressure, a major part of cyanide in the environment will partition to air. The atmospheric half-life due to photodegradation alone was estimated to be 1 to 5 years dependent on the ˙OH concentration. Absorption of HCN by oceans is the major clearance mechanism from air, probably due to the under-saturation of the oceans. This results in an overall atmospheric half-life between 2 and 6 months.

Aquatic fate

The overall rate of cyanide removal from water bodies is governed by the species of cyanide present and the local conditions in the water body. Removal of free cyanide occurs mainly by a combination of complexation, biodegradation, hydrolysis and volatilisation. Volatilisation will prevail if the air is under-saturated with HCN compared to the level in the aquatic compartment. To a minor extent, cyanides may be oxidised to cyanates, which hydrolyse to ammonia and bicarbonate, or in the presence of sulphur are converted into thiocyanates, which are relatively stable in the environment. Photolysis plays a minor role.

Terrestrial fate

The fate of cyanide in soil is the result of a complex interaction of different physical, chemical, physico-chemical factors (e.g. pH, volatilisation, water content, content of sulphur compounds, presence of complexing agents, equilibrium between free and complex cyanide, absorption), and biological and biochemical factors (e.g. presence of organisms with the capacity to metabolise cyanide, toxicity). Whether volatilisation, adsorption, complexation or biodegradation play the major role in a particular soil environment depends on the balance between these factors.

11.1.2 Biodegradation and metabolism in the environment

Cyanide can be metabolised by a wide variety of organisms including bacteria, fungi, arthropods and plants using a number of different pathways (Section 4.3.4). Non-toxic concentrations of

cyanide can be readily biodegraded, both aerobically and anaerobically. Aerobic degradation yields CO_2 and ammonia (that may be further converted to nitrate or nitrite); anaerobic biodegradation yields ammonia and methane. Degradation of cyanides in sewage treatment plants depends on the availability of adapted (micro-)organisms. Sudden high levels of cyanide in these sewage plants may lead to a loss of viability, while fully adapted sludge may tolerate and degrade concentrations up to 100 to 150 mg CN^-/l with a high degree of efficiency. Vegetation is also able to remove cyanide from water or soil. Initial data indicate that cyanide-tolerant plants may be used for the remediation of cyanide-contaminated soil.

11.1.3 Effects on organisms in the environment

Aquatic

Acute toxicity of cyanides to aquatic organisms such as fish, invertebrates or algae has been studied extensively. Acute toxicity to fish as 96-hour LC_{50} values ranged between 27 and 169 µg/l. The lowest value was obtained for rainbow trout (*Oncorhynchus mykiss*) under flow-through conditions. Saltwater species were generally less (factor ≈ 2) sensitive than freshwater species. Acute toxicity to invertebrates showed a wide variation between species and the values in the valid studies ranged from 30 to 2,000 µg/l. The lowest reliable EC_{50} value for *Daphnia magna* was 40 µg/l. The lowest value obtained for the saltwater crab *Cancer irroratus* was 4 µg/l, but for all other species of the genus *Cancer*, EC_{50} values above 30 µg/l were reported. The EC_{50} (24- or 96-h) values for algae ranged from 45 to > 500 µg/l (Section 6.2).

In chronic toxicity studies, reliable NOEC values ranged from 1 to 29 µg/l for fish, 3.9 to approximately 30 µg/l for invertebrates and 3.9 to 700 µg/l for algae.

Terrestrial

The toxicity of cyanides to wildlife was reviewed in Section 6.3.2. Birds, in particular Mallard ducks (*Anas platyrhynchos*), seem to be the most sensitive group or species. The toxicity of cyanides will, however, depend on the dose rate and the conditions of exposure. The steep dose-response curve and the relative quick recovery after sublethal doses of cyanide that were observed in 5 day studies with Mallards are in accordance with the results of studies in other experimental animals and confirm the predominance of acute toxic effects over possible effects after repeated exposure. Although a clear NOAEL could not be established in the existing studies, the LOEL of 21.2 mg CN^-/l based on reduced food and water intake of Mallards can be used as a starting point to derive a level that would probably not lead to significant loss of wildlife. For the

field situation, ingestion of contaminated water is considered the main route of cyanide exposure in birds, although inhalation and dermal exposure cannot be excluded.

Terrestrial plants

In the environment, plants or their seeds and tubers may be exposed to cyanide via air and through soil and pore water. On the other hand, certain plants themselves may be sources of cyanide under certain metabolic conditions or when cyanogenic glucosides are metabolised.

By comparison to other organisms, plants seem to be relatively resistant to cyanides (Section 6.3.3). For example, short-term exposure of roots to concentrations in excess of 1 mg CN^-/l in water seems to be tolerated in most cases. Dormant seeds and tubers show an even higher resistance if exposed by air or water. HCN has been successfully used at concentrations in excess of 1,000 ppm (1,124 mg/m^3) for the fumigation (elimination of parasitic insects) of different types of nutrient grain.

Sewage treatment plant organisms

A number of respiration inhibition tests with sewage organisms of different origin have been reviewed (Section 6.1.4). In a comprehensive ring test, the EC_{50} for respiratory inhibition of sludge from municipal treatment plants ranged from 2.0 to 9.2 mg CN^-/l; the average EC_{50} value was 4.9 mg CN^-/l. A study with domestic sewage revealed a NOEC of 0.2 mg CN^-/l. In an industrial treatment plant, the EC_{50} for respiratory inhibition was 12 to 20 mg/l, indicating adaptation to higher levels of cyanide.

11.1.4 PNEC derivation

Derivation of an aquatic PNEC

To estimate a PNEC for aquatic ecosystems, the short-term LC_{50}/EC_{50} values and the long-term sublethal NOECs for the most sensitive species of fish, invertebrates and algae have been listed in Table 61.

Table 61: Lowest effect concentrations per species

Duration of study / Group	Test species	Concentration (µg CN^-/l)
Short-term		**LC_{50}, EC_{50}**
Fish - freshwater	*Oncorhynchus mykiss*	57
- saltwater	*Menidia menidia*	59
Invertebrates - freshwater	*Daphnia pulex*	100
- saltwater	*Cancer irroratus*	5
Algae, plants - freshwater	*Chlorococcales* sp.	45
- saltwater	*Nitzschia closterium*	57
Long-term		**NOEC**
Fish - freshwater	*Lepomis macrochirus*	1 [a]
- saltwater	*Cyprinodon variegatus*	29
Invertebrates - freshwater	*Gammarus pseudolimnaeus*	3.9
- saltwater	*Mytilus galloprovincialis*	3.2
Algae, plants - freshwater	*Scenedesmus quadricauda*	12
- saltwater	*Champia parvula*	3.9
		HC_5
Fresh- and saltwater	Based on 16 NOECs	1.1

[a] Estimated by dividing the lowest test concentration (NOEC < 5 µg/l) by 5

Except for the saltwater crab *Cancer irroratus*, the EC_{50} values of sensitive algae, invertebrates and fish are in the same order of magnitude and around 50 µg/l. The value for *D. pulex* is 2 fold higher, but it is based on a large number of tests under different conditions. For the common test species *D. magna*, one reliable standardised test revealed an EC_{50} of 40 µg/l. The lowest geometric mean LC_{50}/EC_{50} value per species for freshwater species was 45 µg/l for *Chlorococcales* sp. Among saltwater species, *Cancer irroratus* was much more sensitive than other taxa. Even within the genus *Cancer*, the value for *C. irroratus* is very low: Brix *et al* (2000) tested four other *Cancer* species and found mean EC_{50} per species between 68 and 338 µg/l.

Therefore, the LC_{50}/EC_{50} value of sensitive species can be assumed to be around 50 µg/l in most cases. To derive a PNEC based on acute data, the Technical Guidance Document of the EU (ECB, 2003) recommends an application factor of 100 to the lowest species mean if data for more than the base set (algae, *Daphnia*, fish) are available. This large assessment factor for LC_{50}/EC_{50} should also account for the uncertainty about long-term effects if no data are available. To estimate an ecologically acceptable threshold level for short-term effects on survival, a

smaller factor can be applied. Murgatroyd *et al* (1998) proposed a factor of 10, which would result in an acceptable short-term concentration of 5 µg CN^-/l, approximately. This concentration is around 5 times smaller than the lowest LC_{50} reported for salmonids under reasonable worst-case situations (low temperature or low oxygen levels). Considering that the slope of the dose-response curve for lethal effects of cyanides is usually steep, especially for the sensitive rainbow trout (Table 29) and crustaceans, this seems to be a very protective estimation.

For the estimation of a general aquatic PNEC, the available NOECs for sublethal effects were used. When using a deterministic approach by applying a fixed application factor of 10 to the lowest available NOEC, a PNEC of 0.1 µg CN^-/l would have been obtained. This value seemed to be inappropriate because, given the multiple natural sources of cyanide in the environment, it may well be in the range of natural cyanide concentrations. In view of the large amount of data available for different species and the known common mechanism of cyanide action throughout different species and taxa, the Task Force agreed to apply the species sensitivity distribution (SSD) method to derive an aquatic PNEC (Section 6.2.2). In contrast to the deterministic approach, a SSD uses all the information about the species sensitivities and allows estimating threshold values with confidence intervals, which was considered as the most reliable extrapolation method. For cyanides, NOECs for 16 species were identified as relevant. ECB (2003) recommends assessment factors between 1 and 5 applied to the median HC_5, the concentration protective for 95% of the species. On the one hand, some taxa such as insects or macrophytes are not represented in the SSD for cyanide, suggesting a large assessment factor. On the other hand, according to the LC_{50}/EC_{50} values, these taxa are unlikely to be more sensitive than the taxa included in the SSD. Some of the NOECs used were only estimated from the LOEC, if a significant effect was observed at the lowest test concentration. This leads to a more conservative assessment, because otherwise these data would have been ignored and higher NOECs for these species would have been used. Based on these considerations, the calculated SSD is thought to be conservative and to justify the application of an assessment factor of 1. No indication was found for principal differences between the sensitivities of fresh- and saltwater species to cyanide. Therefore, the SSD also using the data about saltwater algae and molluscs is taken as the most reliable one.

Based on the SSD of the NOECs for sublethal effects on freshwater and saltwater species in laboratory studies (with nominal concentrations), the general aquatic PNEC for free cyanide is suggested to be 1 µg/l.

PNEC soil

Based on a NOEC for terrestrial plants of 1 mg/l (roots in water) and the fact that a number of soil micro-organisms are highly resistant to cyanides (EC_{50} for *Bacillus subtilis* 2,600 mg CN^-/l),

a PNEC soil was derived by dividing the plant NOEC by a factor of 100. This yields a PNEC soil of 10 µg CN^-/l, equivalent to approximately 7 µg CN^-/kg soil.

Tolerable water concentration for wildlife

Applying an assessment factor of 10 to the LOEL of 21.2 mg CN^-/l for Mallard ducks results in a concentration of about 2 mg CN^-/l. This may be a realistic estimate of the concentration that should protect wildlife living around cyanide sources. Based upon the available data, avian species appear to be of comparable sensitivity to rodents (Section 8.1 and 8.3).

PNEC Sewage treatment plant

As a considerable adaptation capacity of sewage sludge to cyanide has been observed in different studies, it seems reasonable to derive 2 PNEC values, one for municipal STPs and one for industrial STPs with adapted micro-organisms.

Using the data from respiration inhibition tests of municipal treatment plants a PNEC can be derived from the typical EC_{50} of 4.9 mg CN^-/l (Section 6.1.4) by dividing it by a factor of 100. This yields a PNEC for municipal treatment plants of 50 µg/l. For industrial treatment plants a PNEC of 120 to 240 µg/l can be derived from the EC_{50} of 12 to 24 mg/l divided by a factor of 100 (all calculations according to EU, 2003).

11.1.5 Summary and conclusions

The PNEC values of free cyanide for the different environmental compartments are summarised in Table 62.

Table 62: Environmental PNECs

Compartment	Endpoint	PNEC as free cyanide (µg CN^-/l)
Aquatic	Acute	5
	Chronic	1
Soil		10 (7 µg/kg)
Wildlife (drinking water)		2,000
STP	Municipal	50
	Industrial	120 - 240

When comparing the PNEC with environmental cyanide levels for risk assessment purposes, it should be realised that the PNECs are based on free cyanide and on laboratory experiments where free cyanide concentrations were maintained. Under environmental conditions, cyanide is frequently found in complex forms. Only the free cyanide level should be compared to the PNEC. This might pose a number of analytical problems that need to be resolved on a case-by-case basis.

11.2 Human health

11.2.1 Toxicokinetics

Cyanide is absorbed quickly via the oral, dermal and inhalation routes. By inhalation, approximately 50% of the inhaled dose is retained in the respiratory tract and subsequently absorbed. After oral uptake, only a small part of the dose reaches the blood due to first-pass metabolism by the liver. Studies on the distribution of cyanide in the body revealed that 50% of the absorbed dose was found in the blood, 25% in muscles and 25% in all other organs together. In mammals, the majority of HCN is metabolised to thiocyanate. Blood thiocyanate levels reflect the balance between thiocyanate formation and excretion while urinary levels roughly follow the concentration of thiocyanate in serum. The average distribution volume of thiocyanate was 0.25 l/kgbw and the elimination half-life in healthy humans was 2.7 days. Excretion of cyanide occurs via exhalation and urinary excretion of thiocyanate.

An understanding of the toxicokinetics of cyanide and thiocyanate is crucial for the understanding of the acute and long-term toxicity and their inter-relationship.

Mode of action

The acute effects of cyanide poisoning are related to its inhibition of cytochrome oxidase aa_3 and the cascade of events leading to cytotoxic anoxia and cell death. The tissues that are most sensitive are those with the highest energy demand, i.e. brain, heart and testes.

The effects due to repeated and chronic exposure to sub-acutely toxic levels of cyanide, such as those on the thyroid and related hormones, are mediated via thiocyanate. Thiocyanate competitively inhibits iodine uptake by the thyroid, leading to decreased thyroid hormone production. Through a compensatory increase in TSH secretion and up-regulation of the thyroid, thiocyanate can induce goitre, particularly in iodine deficient individuals. For this reason, knowledge of the dietary and iodine status of subjects is critical to an understanding of significance to humans of effects observed on the thyroid and related hormones.

When designing toxicity studies in animals, the steep dose-response curve for acute toxicity/lethality makes the selection of dose levels very challenging, especially when the objective is to achieve significant signs of toxicity, while obviously avoiding acute lethality. This is very evident in the repeated-dose inhalation studies with ACH but can also be observed in studies that use other routes or durations. This practical experience in animal studies can be applied to interpreting studies, worker health studies and clinical case studies, such as reports of successful treatment of acute cyanide poisoning. For the same reasons it is often difficult to differentiate between effects due to acute high-exposure from effects that should be attributable to repeated low concentration exposure.

Acute toxicity

Cyanides and ACH are very toxic by the oral, dermal and inhalation routes. On a molar basis, the toxicity of the HCN, NaCN, KCN and ACH are very comparable. The acute oral toxicity (LD_{50}) of the three cyanides in starved rats was 0.127, 0.104 and 0.149 mmol/kgbw (3.3 to 3.9 mg CN^-/kgbw), respectively. The acute LD_{50} for ACH in unstarved rats was 0.188 mmol/kgbw (16 mg/kgbw). The acute dermal LD_{50} of the cyanide solutions in rabbits (intact skin) was between 0.24 and 0.343 mmol/kgbw (6.2 to 8.9 g/kgbw). For ACH, dermal LD_{50} values of 0.188 and 10 mmol/kgbw were reported. The studies differed in the exposed surface area and illustrate the importance of the exposed area for skin absorption. Abrasion of the skin enhanced dermal toxicity. In humans, the average lethal dose after oral exposure is about 1.5 mg/kgbw. The dermal LD_{50} has been estimated to be about 100 mg/kgbw. The acute inhalation toxicity is a function of the body weight of the exposed species and the time of exposure. From the available acute toxicity data via the inhalation route in different species the Task Force derived the following probit equation for the function of body weight.

$$\text{Probit} = 1.133 \times \ln C + 0.413 \times \ln t - 0.108 \ln bw - 1.631 \quad \text{..... (Eq. 40)}$$

Where C = concentration, t = time

When applied to humans this is in good agreement with the reported lethality data.

For the relationship of exposure concentration and time of exposure for humans the following equation was derived.

$$\text{Probit} = 2.81 \times \ln C + 1.37 \times \ln t - 15.5 \quad \text{..... (Eq. 41)}$$

Where C = concentration, t = time

The results for different exposure times also correlate well with human experience.

Using this equation, a human LC_{50} value of 483 mg CN^-/m^3 (10-minute exposure) or 202 mg/m^3 (60-minute) and an LC_{01} of 211 mg/m^3 (10-minute) or 88 mg/m^3 (60-minute) were derived. These values are in good agreement with the human experience data of Bonsall (1984) who reported death after exposure to 203 mg/m^3 for 10 minutes or 121 to 152 mg/m^3 after 30 to 60 minutes.

Skin and eye irritation studies as well as skin sensitisation studies have not been conducted, as the standard amount of cyanide applied in these studies is already lethal. The ocular toxicity was reported to be between 0.039 (HCN) and 0.21 (KCN) mmol/kgbw.

11.2.2 Repeated-dose toxicity

There exists quite an extensive, but fragmented, database on the health effects of cyanides. Numerous studies in animals have been conducted using different species, durations, routes of exposure and form of cyanide (salts, HCN, ACH and cassava) (Chapter 8). The studies in humans include cross-sectional worker health studies from several industries as well as epidemiological studies of cohorts consuming cassava rich diets (Chapter 9). In principle, because cyanide is rapidly absorbed via all routes of exposure (Section 8.1, 8.2, 8.3) and mediates its toxicity through the formation of HCN (Appendix E), the health effects observed in these studies should be relatively independent of route of exposure and broadly consistent between species.

Animal studies

A review of the repeated-dose animal studies reveals two apparent categories of studies (Section 8.3.5).

The first category consists of three 90-day regulatory guideline studies: a drinking-water study in rats and mice (Hébert, 1993) and an inhalation study with ACH in rats (Monsanto, 1984), and a 1-year dietary study in rats (Philbrick *et al*, 1979). In these studies, no effects on thyroid weight or histopathology were observed up to the highest dose tested. Indeed, in the Monsanto study the NOAEL for thyroid effects appeared to be higher than that for general toxicity and was limited by acute toxicity. Only effects on the reproductive organs were observed in one study (Hébert, 1993) at doses below that which resulted in reduced food intake and reduced body-weight gain. The NOAELs from these studies were broadly consistent, i.e. 25.6 mg CN^-/kgbw/d in mice and 12.5 or 10.4 mg CN^-/kgbw/d in rats, respectively, and appear to offer the most robust basis for determining a threshold for chronic toxicity in humans.

The second category consists of a 24-week dietary study in miniature pigs (Jackson, 1988a) and a 14-week dietary study in dogs (Kamalu, 1991, 1993; Kamalu and Agharanya, 1991; Ibebunjo *et al*, 1992). Those studies reported effects on the thyroid and thyroid hormones at doses that were one order of magnitude lower than those that were apparent NOAELs in the studies in the first category. The effects were thought to be due to a combination of effects, including acute toxicity, dietary (iodide) effects and stress, brought about by inappropriate study design (e.g. housing conditions), rather than being reflective of the true repeat-dose toxicity of cyanide.

From the studies of Leuschner *et al* (1991) in rats and Tewe and Maner (1980) in pigs it can be deduced that serum thiocyanate levels of about 20 µg SCN^-/ml consistently did not have goitrogenic effects on the thyroid, if the diet was not iodine deficient. In the Monsanto (1984) inhalation study, levels up to about 80 µg SCN^-/ml in serum did not induce any effects on the thyroid or thyroid hormone levels. Interestingly, those serum concentrations were similar to the concentration of 20 to 40 µg/ml that was regarded as a human NOAEL for thiocyanate therapy in humans (Barker, 1936; Barker *et al*, 1941) (Section 10.3). In the case of iodine deficiency, the same doses can potentially aggravate the goitrogenic effect as shown by Tewe and Maner (1980) in pigs.

11.2.3 Human experience

The available human studies tend to be of two types, cross-sectional worker health (occupational) studies and epidemiological studies. The main effects described in those studies are effects on the thyroid via the metabolite, thiocyanate, and neurological disorders. Among the few occupational

studies cited by the literature (Section 9.2.1), El Ghawabi *et al* (1975) reported effects on the thyroid and the development of goitre. They related the effects to exposure levels of between 4.2 and 12.4 ppm HCN (4.7 - 13.9 mg/m^3). Leeser *et al* (1990) suggested that exposures of 1 to 3 ppm did not result in these effects. Banerjee *et al* (1997) showed a small change in thyroid hormone levels, compared to controls, but no goitre at serum levels of 316 µmol SCN^-/l (measured by the authors) corresponding to 9.2 mg/m^3 (calculated by the Task Force). However, there was no information on the iodine status of the subjects, pre-exposure thyroid hormone levels or circadian variations.

El Ghawabi *et al* (1975) claimed a relationship between the mild to moderate thyroid enlargement observed in 20 electroplating workers (56%) and exposure to thiocyanate (metabolite of cyanide). Thiocyanate excretion levels were, however, comparable to those of the average person in the French population without any cyanide exposure and thyroid hormone levels were normal. Hygiene monitoring (work place air) data were available, but these were limited to 15-minute samples. However, shift average (8-h TWA) exposure levels extrapolated from urinary thiocyanate levels were up to one order of magnitude lower. Due to the apparent inconsistencies between the data provided, some concern must remain regarding the relevance of these findings.

The only other worker health study to report occupational exposure levels was that of Leeser *et al* (1990). In this study, no adverse clinical effects could be attributed to exposures to cyanides of up to approximately 3 ppm HCN (1.1 - 3.4 mg/m^3) (TWA for a typical 8-hour shift). Assuming light exercise (respiration rate 0.8 m^3/h, 8-hour shift and 70 kgbw), this equates to a body burden of up to approximately 0.31 mg CN^-/kgbw/d.

In the study of Dahlberg *et al* (1984) (Section 10.3), application of thiocyanate (8 mg SCN^-/d) to 37 human volunteers (28 females, 9 males) for 12 weeks did not cause any change in the serum level of TSH, T4 or T3 nor in the ratio T3/T4. The excretion of thiocyanate appeared to be around 18 mg/g creatinine and that of iodine about 200 µg/g creatinine (reflecting proper iodine uptake for thyroid). The daily average urinary excretion of creatinine was about 1 g for females and 1.4 g for males. The excretion of thiocyanate was at least 4 times higher than in the study of El Ghawabi *et al* (1975).

Thyroid size in relation to iodide intake and serum thiocyanate in different human populations

Several scientists have studied goitre in European and African populations in relation to the thiocyanate level in serum and/or the urinary iodide excretion per day (Bourdoux *et al*, 1978; Cliff *et al*, 1986; Knudsen *et al*, 2000, 2002). The definition of goitre as used by different authors is not identical and has been a matter of debate (Delange, 1999). Thyroid enlargement is essentially an adaptive and reversible process. A pathological enlargement of thyroid is defined

as 4 to 5 times the normal thyroid size. The new technique of ultrasonography enables determination of the thyroid size more accurately in comparison with palpation and visible enlargement. With this new technique, differences in volume of a few ml (10% to 20%) can be measured as a significant change. Nevertheless, if the thyroid is visibly enlarged with or without extended neck, the thyroid size has been increased by a factor of 4 to 5 and is called goitre (Delange 1999; Knudsen *et al*, 2002). Knudsen *et al* (2000) defined the diagnosis 'thyroid enlargement' by a thyroid volume of more than 25 ml in men and more than 18 ml in women.

Thiocyanate is a competitive inhibitor of iodide absorption by the thyroid. The effect of thiocyanate is compensated for by an increase in TSH release from the pituitary in order to maintain the appropriate T3 and T4 thyroid hormone levels in the blood. This is achieved by increasing the thyroid volume by increasing the thyrocyte volume.

Some authors have correlated increased incidences of thyroid enlargement to heavy smoking, in particular when combined with iodine deficiency (Barrère, 2000; Knudsen *et al*, 2002). However, these studies often did not provide information on the corresponding thiocyanate levels. Information on thiocyanate serum levels in smokers can be drawn from large studies that have determined serum thiocyanate concentrations in smokers and non-smokers in order to get an objective measure of cigarette consumption (Scheuermann *et al*, 1991 cited by Knudsen *et al*, 2000, 2002; Tsuge *et al*, 2000).

Table 63 provides an overview of data from the above studies on measured levels of thiocyanate in serum and urinary iodide excretion in relation to the incidence of goitre.

Table 63: Thiocyanate in serum, iodide in urine and goitre incidence

Population	SCN^- in serum (µmol/l)	I^- excreted in urine (µg/d)	Goitre incidence (%)	Reference
Ubangi school	44.8 ± 5	11.5	92.4	Bourdoux *et al*, 1978
Ubangi rural	183 ± 12	13.5	81.6	Bourdoux *et al*, 1978
Belgian controls	37.9 ± 4	51.2	3 [a]	Bourdoux *et al*, 1978; Hennart *et al*, 1982
Mozambique	250 ± 24	106 ± 10	1.1, slight thyroid hormone level changes	Cliff, 1986
Denmark	85 ± 40 [b]	45	3.9	Knudsen *et al*, 2000
Denmark	85 ± 40 [b]	61	2.5	Knudsen *et al*, 2000
Danish non-smokers	55 ± 30 [c]	45, 61	1.1 [d]	Knudsen *et al*, 2002
Danish heavy smokers	150 ± 50 [e]	45, 61	4.0 [d]	Knudsen *et al*, 2002
Swedish volunteers	68 ± 7	200	0, thyroid not affected	Dahlberg *et al*, 1984
Swedish volunteers	134 ± 7	200	0, thyroid not affected	Dahlberg *et al*, 1984
Electroplating workers	≤ 29 [f]	Not stated	0, thyroid enlargement	El Ghawabi *et al*, 1975
Controls	90.8 ± 9	Not stated	0	Banerjee *et al*, 1997
Electroplating workers	316 ± 15	Not stated	0, thyroid hormone changes slight, but significantly different from controls	Banerjee *et al*, 1997

[a] Estimated by Hennart *et al*, 1982

[b] Measured values of Scheuermann *et al* (1991 as cited) combined male and female smokers and non-smokers, assumed to be representative also for the Danish population

[c] Measured values of Scheuermann *et al* (1991 as cited) combined male and female non-smokers, assumed to be representative also for the Danish population

[d] The association between smoking and goitre was more pronounced in areas with higher iodine deficiency

[e] Measured values of Scheuermann *et al* (1991 as cited) combined male, female, smokers > 20 cigarettes/d, assumed to be representative also for the Danish population

[f] Calculated from the maximum amount excreted in urine: 9 mg SCN^-/d ≈ 0.13 g/kgbw/d, assuming 70 kgbw. Residence time of SCN^- is 3.9 d, thus body burden is 3.49 × 0.13 = 0.5 mg/kgbw/d. Assuming distribution volume of 0.25 l, the SCN^- level is 0.5/0.25 = 2 mg SCN^-/l serum = 29 µmol/l (molecular mass of SCN = 58.1)

Bourdoux *et al* (1978) observed almost the same low thiocyanate levels in serum of Ubangi school girls in Zaire (present Democratic Republic of Congo) and Belgian (Brussels) controls, but at Ubangi school goitre incidence was 92.4% and in Brussels 3% (estimated). There was a

considerable difference in iodine excretion, so lack of iodine was the most probable explanation. A low iodine excretion also occurred in Ubangi rural villages. In rural Mozambique, Cliff *et al* (1986) found very high thiocyanate levels in serum, but almost no goitre (1.1%), probably due to an adequate iodide supply. The authors made a comparison between the population of Zaire and that of Mozambique. In the cassava-eating population of the Ubangi region of Zaire, the prevalence of endemic goitre was reported to be between 60 and 70% (Bourdoux *et al*, 1978), but the excretion of thiocyanate was even lower than in the study population of Mozambique. The authors (Cliff *et al*) concluded that the Mozambique population had adapted to the heavy thiocyanate exposure and that adequate iodide intake, in addition to this adaptation, had protected against the goitrogenic effects of thiocyanate. It was concluded that a serum concentration of 250 µmol/ml (14.5 µg/ml) in the absence of iodine deficiency did not lead to an increased incidence of goitre, but to small changes in thyroid hormone levels that were considered by the authors as adaptive changes.

Knudsen *et al* (2000) studied two Danish cohorts with different iodine excretion levels. Both cohorts were iodide deficient. They observed a higher incidence of thyroid enlargement with decreasing urinary iodide excretion. Knudsen *et al* (2002) found an association between smoking and thyroid enlargement that was more pronounced at higher iodine deficiency.

From Table 63, it might be concluded that it is not possible to draw a simple correlation between the incidence of goitre and blood thiocyanate levels without considering the impact of dietary iodide. This general conclusion is consistent with the work of Hennart *et al* (1982), who suggested that thyroid effects were critically dependent on the ratio of iodine and thiocyanate intake and proposed that a urinary iodine-thiocyanate ratio (as µg I^-/mg SCN^-) of greater than 4 would indicate no concern for thiocyanate-induced goitre (Section 9.2.2).

In this connection, the high variation of serum thiocyanate in non-smokers should also be noted (55 ± 30 µmol/l, Scheuermann *et al*, 1991 cited by Knudsen *et al*, 2002). This wide distribution means that the range of measured data is between 18 and 128 µmol/l serum (i.e. 2.5 and 97.5 percentile, respectively). This high variation obviously did not lead to thyroid malfunctioning. The reason for this high variation was probably related to diet.

In order to better define the borderline between adaptive and pathological changes in thyroid size in connection with thiocyanate exposure, the Task Force compared the reported changes in thyroid size and related them to the serum thiocyanate levels and the iodine status. The normal thyroid size in a population with borderline iodine deficiency (70 µg iodide/l urine) was reported to be about 12 ml for men and 10 ml for women (Knudsen *et al*, 2000). Barrère *et al* (2000) observed a strong relation between thiocyanate level in the urine and the thyroid size in the healthy French population with borderline iodine deficiency. The thiocyanate level in serum was strongly related to smoking. These authors developed a regression equation which may illustrate

the increase of thyroid size with increasing urinary thiocyanate excretion under the condition of borderline iodine deficiency (Table 64).

Table 64: Effects on thyroid size at different thiocyanate and iodine levels

Population	SCN^- in serum (µmol/l)	I^- excreted in urine (µg/d)	Thyroid size (ml)	Reference
Non-smokers	55 ± 30 [a]	45	13.2 ± 0.6	Knudsen *et al*, 2002
Heavy smokers	150 ± 50 [b]	45	17.1 ± 1.1	Knudsen *et al*, 2002
Non-smokers	55 ± 30 [a]	61	11.6 ± 0.5	Knudsen *et al*, 2002
Heavy smokers	150 ± 50 [b]	61	14.1 ± 0.8	Knudsen *et al*, 2002
Healthy males	40 [c]	78 - 86	13.3 [d]	Barrère *et al*, 2000
Male smokers	130 ± 50 [e]	78 - 86	15.1 ± 0.9	Barrère *et al*, 2000
Healthy males	316 ± 15	-	16.4 [d]	Banerjee *et al*, 1997

[a] Measured values of Scheuermann *et al* (1991 as cited) combined male and female non-smokers

[b] Measured values of Scheuermann *et al* (1991 as cited) combined male and female smokers (> 20 cigarettes/d)

[c] Derived from the urinary thiocyanate levels for a 70 kgbw male (using the residence time of 3.9 d, a daily urinary volume of males of 1.4 l and a distribution volume of 0.25 l/kgbw)

[d] Thyroid size estimated by means of regression equation of Barrère *et al*, 2000

[e] Measured values of Scheuermann *et al* (1991 as cited) combined male and female smokers

It seems that small changes in iodide supply (assumed to be in equilibrium with urinary excretion) have a measurable impact on thyroid size (Tables 63 and 64). If the urinary iodide excretion is less than 20 µg/d, the incidence of goitre increases to more than 60% (Table 63). The influence of thiocyanate on the thyroid size is marginal in comparison with lack of iodine. The data of Knudsen *et al* (2002) seem to suggest that, in heavy smokers (> 30 - 40 cigarettes/d), serum thiocyanate levels of 150 ± 50 µmol/l led to an increase in thyroid size. In non-smokers, similar serum thiocyanate levels resulted in increases to about 14 ml in the case of a mild iodine deficiency and to 17 ml in the case of moderate iodine deficiency. The latter group would just reach the borderline of a normal adaptive response in the case of females (no gender differentiation was made in the publication). Interestingly, the authors also reported that the thyroid size in ex-smokers was approximately the same as in non-smokers, indicating reversibility of the effect. The findings of Knudsen *et al* are in accordance with the data of Cliff *et al* (1986) who reported small adaptive changes in thyroid hormones, but no increased goitre incidence, in a cassava-eating non-iodine deficient population with serum levels of about 250 µmol SCN^-/l (14.5 µg/ml). They also agree with the data of Barrère (2000), who reported an increased thyroid volume of 15.1 ml in male French smokers (compared to 12.1 ml in non-smokers) in a slightly iodine deficient population. According to the criteria above, this would also be regarded as an adaptive response rather than a pathological change.

However, the impact of an increase of thyroid size through thiocyanate exposure will depend on the size of the thyroid before exposure. If the thyroid is small, the increase of volume will not be visible. If, due to lack of iodine uptake, the volume of the thyroid is already 24 ml, then an increase in thyroid size of 25% will increase the volume to 30 ml, which would be diagnosed as a thyroid enlargement. So, increased thiocyanate in serum may increase the incidence of thyroid enlargement and ultimately of goitre at low iodine intake. In the case of sufficient iodine supply, the influence of increased thiocyanate will not result in malfunction of the thyroid pituitary axis. Hence, changes in thyroid size might be regarded as a physiological adaptation to variable levels of thiocyanate in blood.

11.2.4 Neurotoxicity

With regard to possible neurotoxic effects related to long-term cyanide exposure (Section 9.2.2), non-occupational studies report a wide range of subjective symptoms that are not clearly related to cyanide exposure. Only a few published cases have associated cyanide exposure with Parkinson-like symptoms. They are all related to acute over-doses of cyanide, mostly in connection with attempted suicides that were treated in a comatose stage. The symptoms seem to be related to brain damage in the basal ganglia, the globus pallidus and putamen that seem to be the most sensitive regions of the brain with regard to cyanide toxicity. There is no indication from the available data that repeated low-dose exposure to cyanide could have similar effects.

Another neurological disorder, ‘konzo’ or tropical neuropathy, which was associated with cyanide exposure, has been observed in populations eating insufficiently processed cassava as a major constituent of their diet. The disease is characterised by symmetric spastic paresis, sometimes combined with disturbances of vision and speech. Most researchers attribute the cause of konzo to a combination of a low protein diet with a consequent deficiency in sulphur amino acids, vitamin B_{12} deficiency and episodes of high intake of cyanogenic glucosides and elevated cyanide blood levels close to acutely toxic levels. It can be concluded that neurological diseases related to diets consisting of cassava with a high content of cyanogenic glucosides are multifactorial diseases that can only partly be attributed to high cyanide blood levels.

In conclusion, all available data suggest that neurological disorders resulting from cyanide intake are related to episodes of acutely toxic cyanide exposure and most likely not caused by repeated intake of low doses of cyanide.

11.2.5 Genotoxicity

In standard *in vitro* and *in vivo* genotoxicity tests, there was no indication of a genotoxic or mutagenic potential of cyanide. Three of the genotoxicity tests and an aneuploidy test in *Drosophila* indicated that metabolic failure at overtly cytotoxic concentrations might also have affected DNA replication and chromatid separation. This was considered to be a secondary effect of toxicity and was not expected to occur at cyanide concentrations that did not inhibit oxygen-dependent metabolism (Section 8.4).

11.2.6 Chronic toxicity and carcinogenicity

In a limited 2-year study in rats receiving diets fumigated with HCN, a NOAEL of approximately 8.2 mg/kgbw/d (7.9 mg CN^-/kgbw/d) was identified. The study was performed with the maximum tolerated dose level determined in a preliminary study. No treatment-related effects were observed and there was no increase in tumour incidence compared to untreated controls (Howard and Hanzal, 1955) (Section 8.5). Although of limited validity, the results of this study are in accordance with the 90-day repeated-dose studies (Section 8.3.5) and indicate that the NOAEL is probably not decreasing with increasing exposure time. This is in accordance with the mode of action relating to steady-state tolerated levels of cyanide and thiocyanate.

With regard to carcinogenicity there is no concern for this endpoint from the genotoxicity studies.

The only possibility of a non-genotoxic tumorigenic effect could be related to the main metabolite, thiocyanate and its effect on the thyroid. Permanent stimulation of the thyroid in the case of a chronically elevated serum thiocyanate level, in particular when in combination with iodine deficiency, could theoretically also lead to a formation of thyroid tumours. Competitive inhibition of iodine uptake into the thyroid by thiocyanate leads to a reduced formation of thyroid hormones (T3 and T4). This triggers, via a negative feed-back mechanism, the production of TRH that, in turn, induces production of TSH. If the TSH level is chronically elevated, thyroid cell hypertrophy and hyperplasia may result. This may ultimately lead to some potential for neoplasia (Hill *et al*, 1998). Under conditions where thyroid-pituitary homeostasis is maintained, the steps leading to possible tumour formation are not expected to develop and the chances of tumour formation are negligible (Hill *et al*, 1998). Thus, if exposure to levels leading to goitre formation is avoided, there should also be no risk of tumour formation (Section 8.5.1). Furthermore, in the epidemiological studies in areas with endemic goitre and cretinism, no increase in thyroid tumour formation was observed. This indicates that the risk of neoplasia in exposed humans is not significant (Section 9.2.2).

11.2.7 Toxicity to reproduction

A number of non-standard studies were available that related cyanide exposure to reproductive toxicity in particular in the context of exploring the effects of dietary cassava. The only studies that gave a clear indication of the relative sensitivity of the reproductive system were those that have used ACH (Section 8.6). ACH showed no evidence for teratogenic effects following gavage dosing in rats up to and including doses that produced maternal toxicity. In male and female fertility studies in rats, inhaled ACH showed no reproductive effects up to concentrations that were limited by the threshold to acute toxicity (local irritative effects and systemic lethality) as indicated in repeated-dose inhalation studies (Section 8.3.3). Therefore, although cyanide is overtly toxic to the reproductive system, there is no reason to suspect that this system is any more sensitive than other organ systems.

A NOAEL of 100 mg/l (≈ 8.6 and 9.6 mg CN^-/kgbw/d) in male and female mice can be derived from the drinking-water study of Hébert (1993). This is consistent with the NOAEL of 8.2 mg HCN/kgbw/d (7.9 mg CN^-/kgbw/d) in rats from the fumigated diet study conducted by Howard and Hanzal (1955). This value is also essentially the same as the NOEL of 57.2 and 58.6 ppm ACH (≈ 8.4 and 8.6 mg CN^-/kgbw) for reproductive effects in male and female rats, respectively, obtained from the inhalation studies of Monsanto (1985a,b). It provides some confidence that the slight effects observed by Hébert (1993) in male rats were unlikely to be cyanide related. This is further supported by the fact that there was no effect on the testes of rats in a comparable drinking-water study at dose levels up to 56 mg CN^-/kgbw (Leuschner *et al*, 1989a cited by WHO, 1993).

11.2.8 Conclusion

The main features of cyanide toxicity are its high acute toxicity combined with a very steep dose-response curve. Possible chronic toxicity is mediated by thiocyanate, the main metabolite and detoxification product. The main route of exposure in the occupational setting and through environmental exposures (fire) is via inhalation. Dermal exposure and even exposure via the eye can also lead to cyanide poisoning. Several hours of exposure to 18 to 30 ppm HCN (20.2 - 33.7 mg/m^3) have been linked to symptoms that are only slight in severity. At 10 ppm (11.2 mg/m^3) no acute symptoms are observed.

The apparent NOAELs for general toxicity (related to the mechanism of inhibiting oxygen utilisation in particular in organs with high oxygen demand, such as brain and testes) are higher, and approximately double, the acute LD/LC_{50} values reported in rats using the same routes of exposure (Section 8.1). This can be explained by the kinetics of the detoxification reactions. It is understood that sustained, lower-level exposures to cyanide are better accommodated by the

detoxification mechanisms (conversion to thiocyanate) in animals and that acute exposure to levels that are above the detoxification capacity will result in direct HCN-mediated cell toxicity and death, particularly in tissues with a high oxygen demand such as the brain. Hence, by spreading the applied dose over a longer exposure period a higher total dose per day (load) can be tolerated. The derivation of an acceptable chronic level is therefore largely based on the effects of elevated serum levels of thiocyanate. The available 90-day studies in rats via the oral and inhalation routes as well as some information derived from human epidemiology studies and experience with human exposure are a good starting point for the derivation of a safe level for humans. Toxicokinetic considerations and results from a 2-year rat study indicate that an additional factor for duration extrapolation seems to be unnecessary. The data on genotoxicity, consideration of carcinogenicity and toxicity to reproduction indicate that those endpoints are not likely to have a lower effect level than that derived for repeated-dose toxicity.

11.3 Derivation of an acceptable cyanide exposure level

An acceptable cyanide exposure level can be derived in a number of ways, including from acute toxicity data, detoxification rates for cyanide, chronic data from animal studies and human (morbidity) studies on the long-term effects of the major metabolite SCN. This addresses two different endpoints, namely short-term (acute) cyanide toxicity and the long-term (chronic) effects of increased thiocyanate levels on the thyroid.

Using the probit analysis of acute inhalation studies and extrapolation to an 8-hour exposure in humans, an LC_{01} of 32 mg CN^-/m^3 can be derived. Given the steep dose-response curve for cyanide, a factor of 3 on this value should prevent acute toxic effects in humans. This is consistent with a tolerable acute dose level of 10 mg CN^-/m^3 reported frequently in the literature. An additional factor of 2 could be applied to account for other uncertainties (e.g. inter-individual differences) resulting in a tolerable dose level of 5 mg CN^-/m^3.

From studies in human patients, infusion of up to 100 to 150 µg sodium nitroprusside/min (44 - 66 µg CN^-/min) did not lead to any signs of cyanide toxicity (Schulz *et al*, 1982; Schulz, 1984). These authors claimed that chronic exposure above these levels will cause fatal poisoning. Careful statistical analysis of their data shows that mortality may occur at infusion rates of more than 3.7 µg CN^-/kgbw/min. However, infusion of 3.0 µg CN^-/kgbw/min (over a period of 80 minutes) will not result in signs of cyanide poisoning with 95% confidence (CN^- levels in erythrocytes will not be higher than 150 µmol/l with 95% confidence). It should be recognised that extrapolating the data derived with relatively short infusion times (mean 80 min) to longer time periods is not without some uncertainty as steady state (infusion = detoxification capacity) may take some time to establish. A conservative approach might be to derive a short term exposure level based on a detoxification rate of 3 µg CN^-/kgbw/min for 80 minutes. For a

one-hour exposure this would result in a tolerable level of 20 mg/m^3 for a 70-kg individual assuming 50% absorption and an inhaled volume of 1.25 m^3 in 1 hour.

To derive an 8-hour exposure level for cyanide-mediated effects, the dose rate of 0.9 µg CN^-/kgbw/min resulting in no increase in cyanide erythrocyte levels can be used (derived by Schulz *et al*, 1982). This is a conservative estimate because it was derived from data on hospitalised patients (that may be assumed to be more susceptible than the general population). Assuming an inhaled volume of 10 m^3, a body weight of 70 kg and 50% absorption, this equates to an exposure level of 6 mg CN^-/m^3 for an 8-hour continuous exposure. At this level, no cyanide accumulation, and hence no cyanide-mediated effects, should occur. This value is consistent with the estimates derived from the acute toxicity data in experimental animals and with human experience.

Taken together, the data regarding chronic toxicity mediated by thiocyanate from the key studies in animals and from human experience provide a relatively consistent picture. The repeated-dose animal studies of Hébert (1993) and Monsanto (1984) appear to offer the most robust basis for determining a threshold for chronic toxicity in humans. The NOAELs in these studies were broadly consistent, i.e. 25.6 mg CN^-/kgbw/d in mice, and 12.5 or 10.4 mg CN^-/kgbw/d in rats. From the studies of Leuschner *et al* (1991) and Monsanto (1985) in rats and Tewe and Maner (1980) in pigs it can be deduced that serum thiocyanate levels of 345 to 475 µmol SCN^-/l (20 - 80 µg/ml) consistently did not have goitrogenic effects on the thyroid if the diet was not iodine deficient. Those serum concentrations are very similar to the concentration of 345 to 690 µmol/l (20 - 40 µg/ml) that was regarded as a human NOAEL for thiocyanate therapy. Cliff *et al* (1986) found that levels of 250 µmol SCN^-/l serum (14.5 µg SCN^-/ml) did not lead to an increased incidence of goitre in a cassava-eating population that was not iodine deficient. Similar findings were reported by Banerjee (1997) for electroplating workers in which serum concentrations of 316 µmol/l (18 µg SCN^-/ml) did not lead to goitre. Both of the latter populations had slight changes in thyroid hormone levels that can be considered as an adaptive response.

A level of 15 µg SCN^-/ml can be used as a starting point to derive a safe concentration of cyanide for humans exposed at the workplace, using the following equation.

$$[CN^-]\ mg/m^3 = \frac{[SCN^-]\ mg/l \times 0.25\ l/kgbw \times 26 \times 70\ kgbw}{10\ m^3 \times\ 0.5 \times 0.8 \times 58 \times 3.9} \quad \text{..... (Eq. 42)}$$

Where
0.25 l/kgbw = distribution volume of SCN^-
26 = molecular mass CN^-
70 kg = body weight worker
10 m^3 = respiratory volume during an 8-hour working day
0.5 = absorption of cyanide by the inhalation route: 50%
0.8 = conversion of cyanide to thiocyanate: ≈ 80%
58 = molecular mass SCN^-
3.9 = elimination of thiocyanate, half-life = 2.7 d, residence time ($t_{0.5}$/ln 2) = 3.9 d

That would mean that an 8-hour exposure to 7.5 mg/m^3 would not lead to effects on the thyroid in healthy humans with sufficient iodine supply. This calculation does not take into account increased renal elimination at increased plasma levels and background thiocyanate plasma levels. Diet or smoking could contribute considerably to these levels. In a non-iodine-deficient situation, the human organism seems to be able to adapt to increased thiocyanate levels without a thyroid enlargement that would be in the 'goitrous' range. However, in moderately iodine-deficient situations, thyroid enlargement can become goitrous if blood thiocyanate levels above approximately 12 µg/ml (210 µmol/l) are maintained, as they are in heavy smokers.

The level of 7.5 mg CN^-/m^3 may be considered as a NOAEL for long-term effects mediated via thiocyanate in humans with sufficient dietary iodine and normal renal function. An analysis of the acute toxicity data and the human detoxification rates suggests that the tolerable concentration in humans with regard to cyanide-mediated acute toxicity may be of the same order of magnitude.

These data are based on an inhalation absorption of HCN of 50%. Inhalation of cyanide salt dust or aerosols may lead to higher absorption rates due to additional swallowing of soluble cyanide salt. Thus tolerable exposure levels for cyanides may be lower (at maximum a factor of 2, assuming an absorption of 100%).

Monitoring of the urinary iodine-thiocyanate ratio and iodine supplementation would be the solution of choice, thereby ensuring that the iodine-thiocyanate ratio (in µg I^-/mg SCN^-) is above 3 to 4. This would be important for both workplace exposure and other exposures, e.g. cassava diets.

With regard to the general population another sensitive sub-population consists of people with impaired renal function. In patients with renal insufficiency, the thiocyanate half-life has been reported to be as high as 9 days (Schulz *et al*, 1979), i.e. a residence time of 13 days. This would correspond to a tolerable value of cyanide inhalation exposure (8 h) in an iodine sufficient situation for this population of 2.3 mg/m^3.

11.4 Summary and evaluation

There are numerous investigations into the effects of cyanide in animals and these include classical (guideline) inhalation and gavage studies as well as drinking water and feeding studies (Section 8.1 and 8.3). These studies have investigated the acute, subacute and subchronic effects of different forms of cyanides including HCN, NaCN and KCN, as well as ACH and thiocyanate (the primary metabolite). The studies are generally consistent in indicating that the critical effects are acute inhibition of oxidative metabolism and effects on the thyroid through the primary metabolite, thiocyanate.

Acute toxicity is characterised by the inhibition of intracellular oxidative metabolism leading to cytotoxic anoxia and cell death. The tissues that are most sensitive are those with the highest energy demand, i.e. brain, heart and testes. Cyanides are acutely very toxic by all routes. The acute oral toxicity (LD_{50}) of HCN, NaCN and KCN in starved rats was 0.127, 0.104 and 0.149 mmol/kgbw, respectively. The acute LD_{50} for ACH in unstarved rats was 0.188 mmol/kgbw. The acute dermal LD_{50} of cyanide solutions in rabbits (intact skin) was between 0.24 and 0.343 mmol/kgbw. In humans, the average lethal dose after oral exposure is about 1.5 mg/kgbw. The dermal LD_{50} has been estimated to be about 100 mg/kgbw.

The acute inhalation toxicity is a function of the body weight of the exposed organism and the time of exposure. Using probit analysis of the acute inhalation data in different species a human LC_{50} for 60 minutes of exposure of 202 mg/m^3 with an LC_{01} 88 mg/m^3 (60-minute exposure) were derived.

Based on infusion experiments in humans and a cyanide level in erythrocytes that should not exceed 40 µmol CN^-/l for prolonged exposure, an exposure level of 11 mg/m^3 over an 8-hour exposure duration was derived. This level is expected not to lead to cyanide-mediated toxicity.

The steep dose-response curve of the acute toxicity sometimes makes it difficult to set dose levels in repeated dose studies. This is particularly so when using the inhalation route or with bolus dosing in oral studies in order to avoid acute toxicity and lethality while still applying the maximum applicable dose to the test animal. Similarly, in reports of human experience when patients have been successfully treated for acute cyanide poisoning it is sometimes difficult to

differentiate between effects that could be the result of several acute, high-exposure incidents, leading to repeated damage, particularly in the brain, and effects attributable to repeated low-concentration exposure, for example at the workplace.

The normal detoxification route for cyanide, via thiocyanate, has a limited capacity. Consequently, when very high serum levels are achieved, this will lead to overt cyanide toxicity. Furthermore, in the rat these high doses may be detoxified by other routes including cyanate and cysteine formation (Section 7.3). These processes are, in turn, dependent upon dietary protein status.

Many studies in animals appear to be deficient for studying the long-term effects on the thyroid. This may be due to inappropriate study design, dosing regime and animal selection, as well as the absence of critical data on plasma and urinary thiocyanate levels and dietary iodide and protein status. In studies employing OECD protocols these factors were adequately controlled but the standard investigation of clinical chemistry limits their usefulness for the assessment of thyroid-related endpoints. For this reason the Task Force primarily looked towards human data.

There are a small number of reported studies on the effect of occupational exposure to CN^- and these are limited to worker health studies in electroplating, precious metal reclamation and sodium salt manufacturing industries (Section 9.2.1). The level of reported exposure during precious metal reclamation and sodium salt manufacture is relatively low and effects on the thyroid have not been reported, so these studies are of limited use for human health hazard assessment. Effects on the thyroid have been reported in the electroplating industry (El Ghawabi *et al*, 1975; Banerjee *et al*, 1997). The first study (El Ghawabi *et al*, 1975) claimed a goitrogenic effect. However, the urinary thiocyanate excretion levels were directly comparable to those of the normal western population (Barrère, 2000), which indicated that iodine deficiency might have been the cause. The other study (Banerjee *et al*, 1997) showed a small change in thyroid hormone levels, compared to controls, but no goitre. From the serum thiocyanate level, the exposure was calculated to be 7 to 9 mg/m^3. However, there was no information on the iodine status of the subjects.

There exists a further source of literature that describes the effect of thiocyanate on the thyroid in humans. This includes nutritional studies, epidemiological (morbidity) studies as well as clinical volunteer studies that would not normally be identified in a conventional literature search on cyanides (Section 13.3). In contrast to the other studies reviewed, these tend to include measurement of relevant clinical and dietary parameters such as dietary protein and iodide, serum and urinary thiocyanate, and smoking habits. All the nutritional studies are consistent in pointing to the criticality of dietary iodine to understanding the influence of thiocyanate on the thyroid (Section 8.0.2). Indeed, if dietary uptake of iodine was sufficiently high to ensure that urinary iodide was greater than approximately 40 µg I^-/d (Hennart *et al*, 1982), then serum thiocyanate

levels of up to approximately 15 µg SCN/ml did not increase the incidence of goitre (Cliff *et al*, 1986). The latter authors noted small changes in thyroid hormone levels at this concentration, but attributed these to a physiological adaptation process. (The Task Force believed this may also be the case in the study of Banerjee *et al*, 1997 above).

The difficulty in defining a NOAEL for cyanide exposure with regard to possible thyroid effects lies in the great variation in background thiocyanate blood levels, depending on nutrition and smoking status, and the iodine status of the exposed population. Furthermore, the borderline between an adaptive effect on the thyroid, i.e. physiological hormone level and volume changes, is not clear-cut. Thyroid enlargement (goitre) has been defined as a thyroid volume above 25 ml for males and above 18 ml for females (Knudsen *et al*, 2002). Several authors have correlated increased incidences of thyroid enlargement to heavy smoking in particular in combination with iodine deficiency (Barrère *et al*, 2000; Knudsen *et al*, 2002). Thiocyanate serum concentrations have been determined in large studies of smokers and non-smokers to obtain an objective measure of cigarette consumption (Scheuermann *et al*, 1991 cited by Knudsen *et al*, 2002; Tsuge *et al*, 2000). In Section 11.2.3 (following Table 64) it is demonstrated that, taken together, the work of Knudsen *et al* (2002), Cliff *et al* (1986) and Barrère (2000) appears to indicate that serum thiocyanate levels of up to approximately 250 µmol SCN^-/l (≈ 15 µg/ml) lead to an increase in thyroid volume that would be regarded by clinicians as an adaptive response and not goitre, even in populations with slight to moderate iodine deficiency.

A thiocyanate level of approximately 15 µg SCN^-/ml in human serum is also comparable to the levels found not to have goitrogenic effects in animals receiving an adequate iodine diet, namely 18 µg SCN^-/ml in pigs (Tewe and Maner, 1980), and up to 27 µg SCN^-/ml in rats (Leuschner *et al*, 1991). A level of 15 µg SCN^-/ml in serum equates to an inhalation exposure of 7.5 mg CN^-/m^3 in the occupational setting and is consistent with the exposure concentration of 7 to 9 mg CN^-/m^3 that can be derived from the serum levels reported by Banerjee *et al* (1997).

Hence, an airborne concentration of 7.5 mg CN^-/m^3 may be considered as a NOAEL for long-term effects mediated via thiocyanate in humans with sufficient dietary iodine and normal renal function. An analysis of the acute toxicity data and the human detoxification rates suggests that the tolerable concentration in humans with regard to cyanide-mediated acute toxicity may be of the same order of magnitude.

These data are based on an inhalation absorption of HCN of 50%. Inhalation of cyanide salt dust or aerosols may lead to higher absorption rates due to additional swallowing of soluble cyanide salt. Thus tolerable exposure levels for cyanide salt dust may be lower (at maximum a factor of 2, assuming an absorption of 100%).

12. FIRST AID AND SAFE HANDLING ADVICE

12.1 Symptoms of poisoning

The majority of symptoms of acute cyanide poisoning can be explained by the inhibition of the Krebs-cycle causing cellular hypoxia. The symptoms often occur in a biphasic pattern, the time course being dependent on the amount and the speed of absorption of the cyanide. In cases of mild poisoning, the early symptoms can usually be observed (Table 65).

Table 65: Symptoms of cyanide poisoning

System	Early stages	Late stages
Central nervous system	Headache, vertigo, dizziness, drowsiness, nausea	Seizure, coma, paralysis
Respiratory	Throat itching (tingling sensation), dyspnoea, tachypnoea [a]	Hypopnoea, Cheyne-Stokes respiration, apnoea
Cardiovascular	Hypertension, sinus/atrio-ventricular nodal arrhythmias, reflex bradycardia	Hypotension, tachycardia, complex arrhythmias, cardiac arrest
Cutaneous (skin)	Pink colour	Blue colour (cyanosis)

[a] Dyspnoea and tachypnoea are the very early signs of poisoning. They are usually explained by the hypoxic stimulation of the chemoreceptors of the carotid sinus. The metabolic acidosis may also contribute to hyperventilation.

12.2 First aid and medical treatment

The speed and effectiveness of the immediate actions taken and the first aid provided at the scene of the accident typically decide the fate of the patient. There is broad agreement on the immediate measures that must be taken.

12.2.1 Immediate measures for emergency responders and first aid officers

- Raise alarm and call for a doctor. Remove the patient from further exposure without endangering yourself (wear protective clothing and respiratory equipment). Immediately take off contaminated clothing and thoroughly rinse the contaminated area with water. Keep the patient warm (emergency blanket) and at rest.
- In combination with mild poisoning (patient conscious, breathing regular): Administer oxygen through a face mask and keep the patient under observation until the doctor arrives.

- In combination with serious to very serious poisoning (patient unconscious, breathing has stopped, possible inclination to convulse): Apply artificial respiration and oxygen with appropriate mechanical means (hand operated respirator bag). Do not use 'mouth to mouth' or 'mouth to nose'.

12.2.2 Therapy recommendation for the doctor

There is no international agreement on the preferred antidote for cyanide poisoning. Several antidote treatments are used and no attempt will be made to rank their efficacy. All antidote therapies work by liberating cyanide that is bound to the iron (II) complex in cytochrome *c* oxidase. These are presented here according to the mechanisms of antidote action.

Methaemoglobin producers

These drugs work by the induction of methaemoglobin, which binds with cyanide to form cyano-methaemoglobin.

Sodium nitrite

This is the preferred drug for medical treatment in the USA. It acts by formation of methaemoglobin. The proportion of methaemoglobin should be monitored for if large doses of sodium nitrite are administered it can cause a fall in blood pressure. Sodium nitrite is available in the Taylor cyanide antidote package (or kit) in 10-ml ampoules containing 300 mg for *i.v.* administration (Baskin and Brewer, 1997). (This is manufactured in the USA by Taylor Pharmaceuticals, Decatur, Illinois [previously by Eli Lily and Company, Indianapolis, Indiana] or can be supplied by other providers). The effective dosage for an adult is 300 to 600 mg (Hall, 1987). A single dose is sufficient to raise the methaemoglobin level to 20% in an adult, and a second dose, up to half as large as the initial one, can be given. Methaemoglobin levels should be monitored if possible and kept below 35% to 40%. The range that is associated with oxygen-carrying deficits causes by methaemoglobin itself (Baskin and Brewer, 1997). Treatment with sodium nitrite is typically followed by treatment with sodium thiosulphate (see below).

The preparation time for injection is approximately 1 minute and the injection time 5 to 15 minutes, with careful monitoring of blood pressure (Baskin and Brewer, 1997). The onset of taking effect has been quoted as 10 minutes (Kiese and Weger, 1969 cited by Degussa, 2000c). Toluidine blue or methylene blue can be given as antidotes in case of false diagnosis and over-dosage. The drug has a shelf-life of 3 years at 25°C (Cummings, 1996).

Amyl nitrite

Amyl nitrite is administered by inhalation rather than *i.v.* injection (as with all other antidotes) and is therefore suitable for administration by non-medical personnel. Amyl nitrite acts by formation of methaemoglobin which, in turn, binds the cyanide ion. Cyanide is then liberated from the methaemoglobin cyanide complex and detoxified, mainly through formation of thiocyanate. The maximum methaemoglobin concentration that can be reached is less than 5%. As Klimmek *et al* (1988) could not find other mechanisms than the formation of methaemoglobin for the antidote effect of amyl nitrite, the detoxification capacity must be considered low. Administration of other antidotes usually follows.

Amyl nitrite ampoules (Taylor cyanide antidote package) contain 0.3 ml and the effective dosage (adult) is achieved with 1 to 4 ampoules by repeated inhalation for 15 seconds, every 30 seconds, for 5 to 6 times per ampoule. The preparation time for use is within seconds making it suitable for immediate treatment. The onset of taking effect is approximately 3 minutes.

Adverse reactions may include hypotension, decrease in arterial oxygen pressure and heart rate (animal experiment: dog). As with sodium nitrite, toluidine blue or methylene blue can be given as antidotes in case of false diagnosis and over-dosage. The drug shelf life is 2 years below 15°C.

Dimethylaminophenol

This is the preferred drug for medical treatment in Germany. In humans, *i.v.* injection of dimethylaminophenol (4-DMAP) (3 mg/kgbw) can produce a level of 15% methaemoglobin within 1 minute. In dogs a dose of 4-DMAP that produces a 30% level of methaemoglobin will save animals that have received 2 to 3 times the LD_{50} of cyanide (Baskin and Brewer, 1997).

Because of the large amount of methaemoglobin formed, a dark blue-brown colour of the skin and mucosa, especially the lips, can be observed immediately after administration of 4-DMAP. This colour must not be mistaken for true cyanosis and does not indicate a clinical deterioration. 4-DMAP should be used in combination with sodium thiosulphate. Repeated doses should be given, if ever, with caution, because the risk of over-dosage (methaemoglobin > 30%) is high. Glucose-6-phosphate dehydrogenase deficiency seems to be an absolute contra-indication.

Before 4-DMAP is used in a patient, it should be quite certain that the patient is poisoned by cyanide. It should only be used if the patient is in a coma. It should not be used in smoke poisoning as carbon monoxide, haemoglobin and methaemoglobin together would not leave enough oxygen carrier capacity.

Each ampoule (Dr. Franz Köhler Chemie, Postfach 1117, 64659 Alsbach-Hähnlein, Germany) contains 5 ml of a 5% solution corresponding to 250 mg 4-DMAP. The effective dosage (adult) is one ampoule. The preparation time for injection is estimated to be less than 1 minute, the injection time also estimated to be approximately 1 minute and the onset of taking effect, 1 to 2 minutes.

Adverse reactions include mild headache and possible tissue necrosis in the case of paravenal injection. Hypotension has been observed in animals, but not in humans.

As with sodium nitrite and amyl nitrite, toluidine blue or methylene blue can be given as antidotes in the case of false diagnosis and over-dosage (Cummings, 1996). The drug shelf life is 4 years at 15 to 25°C, as specified by the supplier.

Cobalt-based drugs

Dicobalt edetate (Kelocyanor)

This is the preferred drug for medical treatment in the United Kingdom. Dicobalt edetate forms a stable complex with cyanide. It has significant toxicity in its own right and should only be used when there is good evidence of cyanide intoxication and must not be given prophylactically (Cummings 1996).

Dicobalt edetate exerts its effect by binding the cyanide ion directly. In animal experiments (dog) the antidote effect has been shown to be superior to that of sodium nitrite/thiosulphate, but 4-DMAP seems to be more effective. Dicobalt edetate can be combined with sodium thiosulphate.

One ampoule (Laboratoires SERB, 53 rue Villiers de l'Isle Adam, 75020 Paris, France; L'Arguenon Limited, Cricketers, Turgis Green, Hook, Nr Basingstoke, RG27 0AH, UK) contains 20 ml of a 1.5% solution corresponding to 300 mg dicobalt edetate. The effective dosage (adult) is 1 ampoule. The preparation time for injection is estimated to be less than 1 minute, the injection time of 1 to 3 minutes and the onset of taking effect at 0 to 2 minutes (in experimental animals: guinea pig).

Adverse reactions include hypotension, collapse and convulsions, facial and pulmonary oedema. Decreased cerebral blood flow has been observed in dogs.

Calcium-disodium-edetate can be given as an antidote in case of false diagnosis and over-dosage. This drug has a shelf life of 3 years at below 25°C.

Hydroxocobalamin

This is the preferred drug for medical treatment in France (Levillain, 1997) and is also used in Germany. Approval for use in the US is currently under discussion. It binds cyanide directly without forming methaemoglobin and is particularly useful under certain conditions such as in fire victims who may already have a decreased concentration of functioning haemoglobin. Hydroxocobalamin is the precursor of vitamin B_{12} and forms cyanocobalamin in the presence of cyanide. It has minimal toxicity or side effects. The currently available preparation under the trade name 'Cyanokit' (Merck Lipha Santé, Lyon, France) contains 2 ampoules of 2.5 g lyophilised cobalamin which can be readily dissolved (in about 1 minute) in 100 ml 0.9% NaCl solution before intravenous injection (Lipha Santé, 1996). The effective dosage (adult) is 5 g (70 mg/kgbw) but this may be repeated according to the degree of intoxication. The onset of taking effect is very short (within ≈ 1 minute in guinea pigs). Adverse anaphylactic reactions have been reported. Antidotes have not proven necessary in the event of false diagnosis and over-dosage. The drug must be protected from light (Cummings, 1996) and has a shelf-life of 2 years at below 25°C, as defined by the supplier.

Sulphur donors

Sodium thiosulphate

This drug has universal recognition for second line use. The utility of thiosulphate is restricted because of its short biological half-life and its small volume of distribution (Baskin and Brewer, 1997). Furthermore, its mode of action is too slow to be used alone in cases of severe poisoning. Sodium thiosulphate acts as a substrate for the enzyme rhodanese, converting cyanide to thiocyanate (Cummings, 1996).

The Taylor cyanide antidote package comprises 50-ml ampoules, each containing 250 mg/ml of sodium thiosulphate. The effective dosage (adult) is 1 ampoule. A second treatment with half of the initial dose may be given. The paediatric dose is 1.65 ml/kgbw. Thiosulphate injection ampoules are also available from other suppliers, e.g. Dr. Franz Köhler Chemie in Germany, American Regent in the USA or Mayne Pharma in Australia.

Although the drug is ready for injection it has to be given *i.v.* over 10 to 20 minutes. The drug has a shelf life of 30 months when stored below 25°C and must be protected from light (Cummings, 1996).

Blood analysis

Blood analysis can be used to determine the severity of exposure (free cyanide and lactate). The analysis should not delay medical treatment.

12.2.3 Summary

According to Baskin and Brewer (1997), the clinical use of most antidotes is based on experiments in animals and on extrapolations made from a small number of clinical cases. Comparing results from animal studies has limitations because of differences in experimental design from one study to another as well as marked interspecies differences in cyanide and drug metabolism. Moreover, the studies were not designed to resemble the typical emergency scenario.

The current situation with divergent use of antidotes for cyanide poisoning, although undesirable, is unlikely to alter in the foreseeable future. This is due to the following factors: the low prevalence of patients treated for cyanide intoxication; the fact that most victims understandably receive multiple therapeutic regimes; the lack of reliable and/or comprehensive data on blood and tissue levels of cyanide; and the limited extent to which comparisons can be drawn between the available animal studies.

Supportive treatment

According to Baskin and Brewer (1997), possibly the most important elements of therapy are general supportive actions which by themselves can affect the recovery of most casualties without further risk from specific antidote therapy. They are the only indicated therapies for casualties of cyanide poisoning who arrive conscious at the emergency medial treatment station.

Lactic acidosis may be treated by *i.v.* administration of sodium bicarbonate and seizures should be controlled by the administration of anticonvulsants.

Because correction of deficiencies in tissue perfusion and oxygenation is the ultimate goal of supportive therapy, it is critically important to maintain an effective cardiac rhythm; this can be accomplished with cardio pulmonary resuscitation.

Oxygen has always been regarded as an important first aid measure in cyanide poisoning. Oxygen accelerates the reactivation of cytochrome oxidase and protects against cytochrome oxidase inhibition by cyanide (Takano *et al*, 1980). Hyperbaric oxygen is recommended for smoke

inhalation victims suffering from combined CO and HCN poisoning. The use of hyperbaric oxygen in pure cyanide poisoning remains controversial.

According to Jacobs (1984) who reported his personal experience of 104 industrial poisoning cases, antidotes were indicated only in severe intoxication cases (deep coma, wide non-reactive pupils, respiratory and circulatory insufficiency). In patients with moderately severe poisoning who had suffered only a brief period of unconsciousness, convulsions or vomiting, therapy consisted of intensive care and *i.v.* sodium thiosulphate. In cases of mild intoxication (dizziness nausea, drowsiness) rest and oxygen alone were used.

Peden *et al* (1986) described 9 patients poisoned by HCN. Three of them were briefly unconscious; their arterial whole blood cyanide concentrations on admission were 3.5, 3.1 and 2.8 mg/l, respectively. The cyanide concentrations in the other cases ranged between 0.93 and 2.6 mg/l. All recovered with supportive therapy alone.

A retrospective study of 25 cases of cyanide poisoning (Bismuth *et al*, 1984 cited by Ballantyne, 1987a) indicated that cardio-respiratory arrests are frequent (7/25) and often inaugural. Deep metabolic acidosis is the rule and mild intoxications are frequently without symptom.

The present treatment of acute cyanide poisoning relies basically on symptomatic measures with sodium bicarbonate, cardiac massage and, above all, assisted ventilation with 100% oxygen.

Survival depends more on prompt medical care than on the accessibility to sophisticated antidotes.

Conclusion

Treatment of cyanide poisoning includes prevention of further cyanide uptake and supportive care. In severe cases of poisoning, the very early administration of antidotes is mandatory.

All the treatments listed above have been shown to be effective in the management of cyanide poisoning. They all have advantages and disadvantages, whether these lie in their inherent toxicity or difficulty in maintaining the availability of the antidote at the site of potential exposure.

Users should familiarise themselves with these drugs and elect for one regime that they feel is best for their practice and meets their national legal requirements. Once made, this decision should be changed only with great deliberation. Unjustified or frequent changing of

recommended therapies could lead to confusion and, ultimately, to a lack of public confidence (Cummings, 1996).

12.3 Safe handling

12.3.1 Safety at work

As cyanides are very toxic substances, staff need to be informed and trained thoroughly in handling them. The required information for safe handling of cyanides is available for all downstream users as well as for the producers themselves in the safety data sheet (SDS) for the respective substance. As SDSs also reflect the present status on important safety information and regulations, only the most recent SDS should be taken as a reference source.

In all situations where exposure to cyanides is possible, adequate personal protective equipment must be used. When working with cyanides, alarm mechanisms should be installed to ensure that there is immediate help in case of an accident. For the same reason, nobody should work alone with cyanides in a closed room. All employees should receive training repeated at regular intervals. Any areas that have become contaminated with cyanide have to be washed thoroughly with water that, in turn, must be recycled to the process or decontaminated (CEFIC, 2006).

Maintenance

When isolating pipelines or piping for removal, the use of line blinds or other positive means of disconnection is recommended. Tanks, pumps, lines and valves should always be drained and thoroughly flushed free of cyanide with water or steam without exposing people to contaminated steam or washing water. Steel might absorb cyanide from solution and may slowly release cyanide. Frequent monitoring is therefore required to ensure that previously decontaminated steel work is still free of cyanide (CEFIC, 2006).

Respiratory protection

At least full face mask with the appropriate filter should be used routinely. In the event of an emergency a self contained breathing apparatus is obligatory (CEFIC, 2006).

Eye protection

Close fitting goggles or full face mask should be used (CEFIC, 2006).

Protective clothing

CEFIC (2006) recommends rubber or plastic gloves, rubber boots, overalls and hood with complete protection of the head, face and neck.

Contaminated clothes should be changed immediately.

12.3.2 Storage safety

Cyanides must be stored in a separate, locked, well ventilated room under cover and under strong supervision. Detailed records of removal and receipt of cyanides have to be kept.

Cyanides should never be stored together with acids, acid salts, weak alkalis, oxidising materials or items destined for human consumption. They should be stored separately from flammable materials. Details of storage are subject to national regulations (CEFIC, 2006).

12.3.3 Fire safety and extinguishants

In the case of fire, self-contained breathing apparatus and full-protective suit have to be worn. Sufficient retaining facilities for the water used to extinguish fire should be available as the water should not enter drainage systems, soil or natural water bodies. Contaminated fire extinguishing water and fire residues must be disposed of in accordance with local regulations (Degussa, 1999, 2000a,b).

Suitable extinguishing media include, for alkali cyanides, an alkali powder quenching agent (Degussa 2000a,b) and for HCN, water, mist or carbon dioxide (Degussa, 1999).

Extinguishing media that must not be used for alkali cyanides are water, foam, carbon dioxide, acid quenching agents and acid powder quenching agents (Degussa 2000a,b).

12.3.4 Protection against fire and explosion

For HCN, only explosion-proof equipment should be used. Keep away from sources of ignition, direct sunlight and heat. Avoid electrostatic charges (Degussa, 1999).

12.4 Management of spillage and waste

Solid spills and wastes may be picked up mechanically (full protective equipment is needed as outlined above) and collected in suitable containers. The material can be re-used or disposed of in accordance with local regulations. Solutions should be absorbed with liquid binding material (e.g. inert adsorbent, diatomaceous earth or acid neutraliser) then picked up mechanically and disposed of in accordance with local regulations (Degussa, 1999, 2000a,b; CEFIC, 2006). Spillages and wastes can be decontaminated by detoxification procedures (see below).

Whenever cyanides are manufactured or used, effluents and wastes containing various amounts of cyanide are produced. Because of the high toxicity of cyanide to all forms of life, the effluents and wastes must be treated to reduce the cyanide content to concentrations that are acceptable with the regard to the particular environmental conditions (Spector, 1956; Doudoroff, 1976, 1980; US-EPA, 1980; Huiatt *et al*, 1983). Depending on the quantity and type of cyanidic waste, various detoxification methods are used (Dodge and Reams, 1949; Reed *et al*, 1971 cited by Young and Jordan, 1995; Ingles and Scott, 1981). In addition to effectiveness and cost of treatment, the formation of undesirable by-products and additional salting of the wastewater are factors of growing importance in choosing an effluent-treatment method.

Heavy metals may be precipitated and separated as hydroxides, carbonates, or sulphides, when complex heavy metal cyanides are oxidised. Wastewater treatment can be carried out batch-wise or continuously, and the process may be monitored and controlled automatically (only possible if there is no big change in the matrix).

Detoxification of ACH wastes

Dilute solutions can be treated either with caustic soda and ferrous sulphate (this is an elimination not a detoxification), or sodium carbonate (the same) or hydrogen peroxide before controlled disposal.

The ACH process is essentially closed and does not produce any continuous liquid waste streams. However, aqueous waste streams can occasionally arise from plant maintenance and decontamination activities.

Effluent containing ACH is normally burnt in an incinerator suitable for toxic waste and energy recovery, or recycled into methyl methacrylate plants. (Unlike cyanides, treatment of ACH with hypochlorite in alkaline conditions would promote chloroform formation).

12.4.1 Chemical treatment of cyanide-containing wastes

The most common methods for the treatment of cyanide-containing wastes are the oxidation to cyanate by reaction with chlorine or hydrogen peroxide.

Chlorination

Alkaline chlorination is the most frequently used process for the treatment of effluents containing < 1 g CN^-/l (Beevers, 1972; White, 1972). The treatment can be carried out with chlorine and alkali [NaOH, $Ca(OH)_2$] or with ready-made hypochlorite solutions that contain about 12% NaOCl. At first, toxic cyanogen chloride is formed, which is hydrolysed quickly to cyanate and chloride at pH > 11 (Stumm *et al*, 1954):

$$CN^- + OCl^- + H_2O \rightarrow ClCN + 2\ OH^- \quad \text{(Eq. 43)}$$

$$ClCN + 2\ OH^- \rightarrow OCN^- + Cl^- + H_2O \quad \text{(Eq. 44)}$$

Hydrogen peroxide oxidation

The use of hydrogen peroxide for cyanide detoxification has increased in recent years because, in this case, the oxidation of cyanide leads directly to cyanate without the formation of toxic intermediates and the by-product chloride:

$$CN^- + H_2O_2 \rightarrow OCN^- + H_2O \quad \text{(Eq. 45)}$$

Other methods of treatment

Another common method is based on the treatment of effluents with a mixture of sulphur dioxide (2.5%) and air in the presence of small amounts of copper salts (> 50 mg Cu/l, also possible with smaller quantities) as catalyst (Devuyst *et al*, 1989; Beevers, 1972):

$$CN^- + O_2 + SO_2 + H_2O \rightarrow OCN^- + H_2SO_4 \quad \text{(Eq. 46)}$$

In the case of highly concentrated cyanide solutions, the volatilisation and catalytic oxidation of HCN after acidification of the solutions can be used. Sometimes, the HCN can be re-used by absorption in alkali metal hydroxide solution and conversion to alkali metal cyanide. Atmospheric oxygen is occasionally used for the destruction of cyanide in weak solutions, in

combination with activated carbon (Wysocki and Höke, 1974), aeration combined with acid and base catalysed hydrolysis processes (Gaudy *et al*, 1982), or micro-organisms (Murphy and Nesbitt, 1964). Removal of cyanides by ion exchange and reverse osmosis does not solve the problem entirely because the concentrates require further treatment.

The hydrolysis of cyanide at 180 to 230°C under pressure can be used for the destruction of simple and complex cyanides, even in high concentrations (Hoerth *et al*, 1973).

The conversion of cyanide to thiocyanate by the addition of sulphur is a process that used to be employed but is now regarded more critically (Dodge and Reams, 1949). This process has the advantage of low reagent costs, but the investment for plant can be high. It is less suitable for seasonal detoxification, since start up and decommissioning can take time.

Very effective oxidation of cyanide to cyanate, carbonate, and nitrogen takes place with ozone (Hoerth *et al*, 1973); however, this requires high capital investment and causes problems with the adaptation to varying oxygen demand.

The conversion of cyanide to ferrocyanide by reaction with iron(II) salts in alkaline solution followed by precipitation as iron (II) ferrocyanide, $Fe_2[Fe(CN)_6]$, at pH 3 to 4 is one of the oldest methods for cyanide removal. However, this should be used only for the removal of complex iron cyanides, if at all, because the sludge must be disposed of in such a manner that water resources are protected. Furthermore, the filtrates must be treated again to destroy the remaining traces of cyanide.

In many cases, low concentrations of complex iron cyanides (e.g. < 20 mg CN^-/l) in effluents discharged to sewage systems are tolerated because they are precipitated in contact with metal salts (e.g. the iron salts present in domestic wastewater) and are separated with the sludge of municipal sewage treatment plants.

Treatment of ACH wastes

ACH can be detoxified by any of the methods outlined previously. Treatment with hydrogen peroxide is the preferred method as alkaline chlorination may result in the formation of chloroform from the reaction of cyanogen and acetone.

Decontamination of tailings ponds

Detoxification of cyanides prior to any discharge into tailings ponds can be performed by one of the methods outlined above. Free and many metal bound cyanides can be oxidised but usually not thiosulphate. As a consequence, most of a pond's cyanide toxicity can be reduced or even eliminated by the application of industrial detoxification processes prior to the discharge into the tailings. During detoxification, the heavy metals are also precipitated as hydroxide or oxides and made water insoluble. This effect reduces the overall toxicity in a pond significantly. In case of a pond rupture, this can be of major importance to minimise negative effects to the environment.

In 2006, the EU adopted a new Mining Waste Directive, limiting the cyanide discharge to a maximum of 50 mg CN^-/l (WAD cyanide at the point of discharge of the tailings from the processing plant into the pond). This value will be enforced from 1 May 2008 and further lowered in two steps to 25 and 10 mg/l at 5 and 10 years, respectively, for existing facilities. New permits granted after 1 May 2008 will allow for 10 mg/l only (CEC, 2006b). In practice, this will mean that the feed to tailings facility needs to have cyanide levels reduced by approximately 90%.

APPENDIX A: SPECIAL ABBREVIATIONS, SYMBOLS, UNITS AND PREFIXES

≈	Approximately
~	Estimated
↓	Decrease, reduction
↑	Increase, elevation
<	Less than
µ-	Micro (10^{-6})
>	More than
≥	More than or equal to
–ve	Negative
+ve	Positive
√	Square root
×	Times, fold

ACH	Acetone cyanohydrin
ADP	Adenosine diphosphate
ALT	Alanine aminotransferase
APV	2-Amino-5-phosphonovalerate
AST	Aspartate aminotransferase
ATP	Adenosine triphosphate
BAF	Bioaccumulation factor
BCF	Bioconcentration factor
BOD	Biological oxygen demand
bw	Body weight
C	Concentration
CAS	Chemical Abstracts Service
CN^-	Cyanide anion
CO	Carbon monoxide
CoR	Code of reliability
d	Day
DIN	Deutsches Institut für Normung
EAT	ECETOC aquatic toxicity (database)
EC	European Commission
EC_{50}	Median effect concentration
EINECS	European inventory of existing commercial chemical substances
EPER	European pollutant emission register
ET_{50}	Median effective time

G-	Giga (10^9)
GC	Gas chromatography
GSH	Glutathione
h	Hour
HC_5	Hazardous concentration for 5% of species
HCN	Hydrogen cyanide
hPa	Hectopascal
HPLS	High performance liquid chromatography
IC_{25}	Inhibition concentration that causes a 25% reduction
i.p.	Intraperitoneal
IP_3	Inositol triphosphate
i.v.	Intravenous
ISO	International Organization for Standardization
IUPAC	International Union of Pure and Applied Chemistry
J	Joule
k-	Kilo (10^3)
KCN	Potassium cyanide
K_{oc}	Partition coefficient (organic carbon/water)
K_{ow}	Partition coefficient (octanol/water)
l	Litre
LAD	Laboratory animal diet
LC_x	Lethal concentration to x% of the population
LC_{50}	Median lethal concentration
LC_{100}	Absolute lethal concentration
LD_{50}	Median lethal dose
LETC	Lethal threshold concentration
LOEC/LOEL	Lowest-observed effect concentration or level
ln	Natural logarithm
log	Common logarithm
LT_{50}	Median survival time
M-	Mega (10^6)
m	Metre
m-	Milli (10^{-3})
MATC	Maximum acceptable toxicant concentration
min	Minute
MK-801	(+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohept-5,10-imine maleate
mol	Mole
MS	Mass spectrometry
Mw	Molecular weight (mass)

n	Number
n-	Nano (10^{-9})
NaCN	Sodium cyanide
NAD(P)H	Reduced nicotanimide adenine dinucleotide (phosphate)
NMDA	N-methyl-D-aspartate
NO	Nitric oxide
No.	Number
NOAEL	No observed adverse effect level
NOEC	No observed effect concentration
˙OH	Hydroxyl radical
p-	Pico (10^{-12})
PG	Packing group
pH	–log (H^+ concentration), measure of acidity
pKa	–log (acidity constant), measure of extent of acidity
PKC	Phosphokinase C, protein kinase C
PLA2	Phospholipase A2
PNEC	Predicted no effect concentration
ppb	Parts per billion (10^9), by volume
ppm	Parts per million (10^6), by volume
ppt	Parts per trillion (10^{12}), by volume
ROS	Reactive oxygen species
R-	Risk
s	Second
S-	Safety
S9	Supernatant of centrifuged 9,000 × g liver homogenate
SAA	Sulphur-amino acid
SAFARI	Southern Africa regional science initiative
s.c.	Subcutaneous
SCN^-	Thiocyanate anion
sp.	Species
SOD	Superoxide dismutase
SSD	Species sensitivity distribution
t	Tonne, time
T3	Tri-iodothyronin
T4	Thyroxin
TRACE-P	Transport and chemical evolution in the Pacific ocean
TRH	Thyrotropin releasing hormone
TRI	Toxics release inventory
TSH	Thyroid stimulating hormone
TWA	Time-weighted average

U	Unit
WAD	Weak acid dissociable
wk	Week
y	Year

APPENDIX B: CRITERIA FOR RELIABILITY CATEGORIES

Adapted from Klimisch *et al* (1997)

Code of Reliability (CoR)	**Category of reliability**
1	Reliable without restriction
1a	'Good laboratory practice' guideline study (OECD, EC, EPA, FDA, *etc.*)
1b	Comparable to guideline study
1c	Test procedure in accordance with national standard methods (AFNOR, DIN, *etc*.)
1d	Test procedure in accordance with generally accepted scientific standards and described in sufficient detail
2	Reliable with restrictions
2a	Guideline study without detailed documentation
2b	Guideline study with acceptable restrictions
2c	Comparable to guideline study with acceptable restrictions
2d	Test procedure in accordance with national standard methods with acceptable restrictions
2e	Study well documented, meets generally accepted scientific principles, acceptable for assessment
2f	Accepted calculation method
2g	Data from handbook or collection of data
3	Not reliable
3a	Documentation insufficient for assessment
3b	Significant methodological deficiencies
3c	Unsuitable test system
4	Not assignable
4a	Abstract
4b	Secondary literature
4c	Original reference not yet available
4d	Original reference not translated
4e	Documentation insufficient for assessment

APPENDIX C: CONVERSION FACTORS FOR VAPOUR CONCENTRATIONS IN AIR

Conversion factors for vapour concentrations in air can be calculated from the molar volume of an ideal gas at 0°C: 22.4136 litre.

$$1\ mg/m^3 = 22.4136/Mw \times 1{,}013.25/P \times (273 + T)/273\ ppm \quad \text{(Eq. C.1)}$$

$$1\ ppm = Mw/22.4136 \times P/1{,}013.25 \times 273/(273 + T)\ mg/m^3 \quad \text{(Eq. C.2)}$$

Where Mw = molecular weight (mass), T = temperature (°C) and P = pressure (hPa).

For European standard conditions, 20°C and 1,013.25 hPa (= 1 atm = 760 mm Hg), the formulae become:

$$1\ mg/m^3 = 24.0556/Mw\ ppm \quad \text{(Eq. C.3)}$$

$$1\ ppm = Mw/24.0556\ mg/m^3 \quad \text{(Eq. C.4)}$$

In the USA and other countries 25°C is used, and the formulae are:

$$1\ mg/m^3 = 24.4661/Mw\ ppm \quad \text{(Eq. C.5)}$$

$$1\ ppm = Mw/24.4661\ mg/m^3 \quad \text{(Eq. C.6)}$$

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ECETOC PUBLISHED REPORTS

Monographs

No.	Title
No. 1	Good Laboratory Practice (Published October 1979)
No. 2	A Contribution to Strategy for Identification and Control of Occupational Carcinogens (Published September 1980)
No. 3	Risk Assessment of Occupational Chemical Carcinogens (Published May 1985)
No. 4	Hepatocarcinogenesis in Laboratory Rodents: Relevance for Man (Published October 1982)
No. 5	Identification and Assessment of the Effects of Chemicals on Reproduction and Development (Reproductive Toxicology) (Published December 1983)
No. 6	Acute Toxicity Tests, LD_{50} (LC_{50}) Determinations and Alternatives (Published May 1985)
No. 7	Recommendations for the Harmonisation of International Guidelines for Toxicity Studies (Published December 1985)
No. 8	Structure-Activity Relationships in Toxicology and Ecotoxicology: An Assessment (Summary) (Published June 1986)
No. 9	Assessment of Mutagenicity of Industrial and Plant Protection Chemicals (Published June 1987)
No. 10	Identification of Immunotoxic Effects of Chemicals and Assessment of their Relevance to Man (Published August 1987)
No. 11	Eye Irritation Testing (Published June 1988)
No. 12	Alternative Approaches for the Assessment of Reproductive Toxicity (with emphasis on embryotoxicity/teratogenicity) (Published November 1989)
No. 13	DNA and Protein Adducts: Evaluation of their Use in Exposure Monitoring and Risk Assessment (Published October 1989)
No. 14	Skin Sensitisation Testing (Published March 1990)
No. 15	Skin Irritation (Published July 1990)
No. 16	Early Indicators of Non-Genotoxic Carcinogenesis (Published June 1991)
No. 17	Hepatic Peroxisome Proliferation (Published May 1992)
No. 18	Evaluation of the Neurotoxic Potential of Chemicals (Published September 1992)
No. 19	Respiratory Allergy (Published August 1993)
No. 20	Percutaneous Absorption (Published August 1993)
No. 21	Immunotoxicity: Hazard Identification and Risk Characterisation (Published September 1994)
No. 22	Evaluation of Chemicals for Oculotoxicity (Published November 1994)
No. 23	Receptor Mediated Mechanisms in Chemical Carcinogenesis (Published December 1995)
No. 24	Risk Assessment for Carcinogens (Published July 1996)
No. 25	Practical Concepts for Dose Selection in Chronic Toxicity and Carcinogenicity Studies in Rodents (Published February 1996)
No. 26	Aquatic Toxicity Testing of Sparingly Soluble Volatile and Unstable Substances (Published September 1996)
No. 27	Aneuploidy (Published August 1997)
No. 28	Threshold-Mediated Mutagens - Mutation Research Special Issue (Published January 2000)
No. 29	Skin Sensitisation Testing for the Purpose of Hazard Identification and Risk Assessment (Published September 2000)
No. 30	Genetic Susceptibility to Environmental Toxicants (Published October 2001)

No. 31 Guidance on Evaluation of Reproductive Toxicity Data (Published February 2002)

No. 32 Use of Human Data in Hazard Classification for Irritation and Sensitisation (Published July 2002)

No. 33 Application of Physiological - Toxicokinetic Modelling to Health Hazard Assessment of Chemical Substances (Published February 2003)

No. 34 Toxicogenomics in Genetic Toxicology and Hazard Determination (Published July 2005)

No. 35 Biomarkers and molecular epidemiology (Published August 2006)

No. 36 Environmental Genotoxins in Children and Adults (Published September 2006)

Technical Reports

No. Title

No. 1 Assessment of Data on the Effects of Formaldehyde on Humans (Published January 1979) (Updated by TR No. 6)

No. 2 The Mutagenic and Carcinogenic Potential of Formaldehyde (Published May 1981) (Updated by TR No. 6)

No. 3 Assessment of Test Methods for Photodegradation of Chemicals in the Environment (Published August 1981)

No. 4 The Toxicology of Ethylene Glycol Monoalkyl Ethers and its Relevance to Man (Published June 1982) (Updated by TR No. 17)

No. 5 Toxicity of Ethylene Oxide and its Relevance to Man (Published September 1982) (Updated by TR No. 11)

No. 6 Formaldehyde Toxicology: An Up-Dating of ECETOC Technical Reports 1 and 2 (Published September 1982)

No. 7 Experimental Assessment of the Phototransformation of Chemicals in the Atmosphere (Published September 1983)

No. 8 Biodegradation Testing: An Assessment of the Present Status (Published November 1983)

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No. 10 Considerations Regarding the Extrapolation of Biological Data in Deriving Occupational Exposure Limits (Published February 1984)

No. 11 Ethylene Oxide Toxicology and its Relevance to Man: An Up-Dating of ECETOC Technical Report No. 5 (Published March 1984)

No. 12 The Phototransformation of Chemicals in Water: Results of a Ring-Test (Published June 1984)

No. 13 The EEC 6th Amendment: A Guide to Risk Evaluation for Effects on the Environment (Published March 1984)

No. 14 The EEC 6th Amendment: A Guide to Risk Evaluation for Effects on Human Health (Published March 1984)

No. 15 The Use of Physical-Chemical Properties in the 6th Amendment and their Required Precision, Accuracy and Limiting Values (Published June 1984)

No. 16 A Review of Recent Literature on the Toxicology of Benzene (Published December 1984)

No. 17 The Toxicology of Glycol Ethers and its Relevance to Man: An Up-Dating of ECETOC Technical Report No. 4 (Published April 1985) (Updated by TR No. 64)

No. 18 Harmonisation of Ready Biodegradability Tests (Published April 1985)

No. 19 An Assessment of Occurrence and Effects of Dialkyl-o-Phthalates in the Environment (Published May 1985)

No. 20 Biodegradation Tests for Poorly-Soluble Compounds (Published February 1986)

No. 21 Guide to the Classification of Carcinogens, Mutagens, and Teratogens under the 6th Amendment (Published February 1986)

No. 22 Classification of Dangerous Substances and Pesticides in the EEC Directives. A Proposed Revision of Criteria for Inhalational Toxicity (Published January 1987)

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No. 25 Evaluation of Fish Tainting (Published January 1987)

No. 26 The Assessment of Carcinogenic Hazard for Human Beings exposed to Methylene Chloride (Published January 1987)

No. 27 Nitrate and Drinking Water (Published January 1988)

No. 28 Evaluation of Anaerobic Biodegradation (Published June 1988)

No. 29 Concentrations of Industrial Organic Chemicals Measured in the Environment: The Influence of Physico-Chemical Properties, Tonnage and Use Patterns (Published June 1988)

No. 30 Existing Chemicals: Literature Reviews and Evaluations (Fifth Edition) (No longer available) (Published May 1994)

No. 31 The Mutagenicity and Carcinogenicity of Vinyl Chloride: A Historical Review and Assessment (Published July 1988)

No. 32 Methylene Chloride (Dichloromethane): Human Risk Assessment Using Experimental Animal Data (Published May 1988)

No. 33 Nickel and Nickel Compounds: Review of Toxicology and Epidemiology with Special Reference to Carcinogenesis (Published February 1989)

No. 34 Methylene Chloride (Dichloromethane): An Overview of Experimental Work Investigating Species Differences in Carcinogenicity and their Relevance to Man (Published March 1989)

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No. 38 A Guide to the Classification of Preparations Containing Carcinogens, Mutagens and Teratogens (Published July 1990)

No. 39 Hazard Assessment of Floating Chemicals After an Accidental Spill at Sea (Published July 1990)

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No. 42 Critical Evaluation of Methods for the Determination of N-Nitrosamines in Personal Care and Household Products (Published February 1991)

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No. 45 Nickel, Cobalt and Chromium in Consumer Products: Allergic Contact Dermatitis (Published March 1992)

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No. 47 EC 7th Amendment 'Toxic to Reproduction': Guidance on Classification (Published August 1992)

No. 48 Eye Irritation: Reference Chemicals Data Bank (Second Edition) (Published June 1998)

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No. 58 Assessment of Non-Occupational Exposure to Chemicals (Published May 1994)

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No. 60 Trichloroethylene: Assessment of Human Carcinogenic Hazard (Published May 1994)

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No. 62 Ammonia Emissions to Air in Western Europe (Published July 1994)

No. 63 Reproductive and General Toxicology of some Inorganic Borates and Risk Assessment for Human Beings (Published February 1995)

No. 64 The Toxicology of Glycol Ethers and its Relevance to Man (Published August 1995) (Updated by TR No. 95)

No. 65 Formaldehyde and Human Cancer Risks (Published May 1995)

No. 66 Skin Irritation and Corrosion: Reference Chemicals Data Bank (Published March 1995)

No. 67 The Role of Bioaccumulation in Environmental Risk Assessment: The Aquatic Environment and Related Food Webs (Published October 1995)

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No. 70 Chronic Neurotoxicity of Solvents (Published February 1996)

No. 71 Inventory of Critical Reviews on Chemicals (Published August 1996) (Only available to ECETOC members)

No. 72 Methyl *tert*-Butyl Ether (MTBE) Health Risk Characterisation (Published June 1997)

No. 73 The Value of Aquatic Model Ecosystem Studies in Ecotoxicology (Published December 1997)

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No. 78 Skin Sensitisation Testing: Methodological Considerations (Published December 1999)

No. 79 Exposure Factors Sourcebook for European Populations (with Focus on UK Data) (Published June 2001)

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No. 81 Human Acute Intoxication from Monochloroacetic Acid: Proposals for Therapy (Published November 2001)

No. 82 Risk Assessment in Marine Environments (Published December 2001)

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No. 84 Scientific Principles for Soil Hazard Assessment of Substances (Published July 2002)

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No. 91 Aquatic Hazard Assessment II (Published November 2003)

No. 92 Soil and Sediment Risk Assessment (Published December 2004)

No. 93 Targeted Risk Assessment (Published December 2004)

No. 94 Whole Effluent Assessment (Published December 2004)

No. 95 The Toxicology of Glycol Ethers and its Relevance to Man (Fourth Edition) Volume I and Volume II Substance Profiles (Published February 2005)

No. 96 Trends in Children's Health and the Role of Chemicals: State of the Science Review (Published June 2005)

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Joint Assessment of Commodity Chemicals (JACC) Reports

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No. 1 Melamine (Published February 1983)

No. 2 1,4-Dioxane (Published February 1983)

No. 3 Methyl Ethyl Ketone (Published February 1983)

No. 4 Methylene Chloride (Published January 1984)

No. 5 Vinylidene Chloride (Published August 1985)

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No. 9 Chlorodifluoromethane (Published October 1989)

No. 10 Isophorone (Published September 1989)

No. 11 1,2-Dichloro-1,1-difluoroethane (HFA-132b) (Published May 1990)

No. 12 1-Chloro-1,2,2,2-tetrafluoroethane (HFA-124) (Published May 1990) (Updated by JACC No. 25)

No. 13 1,1-Dichloro-2,2,2-trifluoroethane (HFA-123) (Published May 1990) (Updated by JACC No. 33)

No. 14 1-Chloro-2,2,2-trifluoromethane (HFA-133a) (Published August 1990)

No. 15 1-Fluoro 1,1-dichloroethane (HFA-141b) (Published August 1990) (Updated by JACC No. 29)

No. 16 Dichlorofluoromethane (HCFC-21) (Published August 1990)

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No. 24 Pentafluoroethane (HFC-125) (CAS: 354-33-6) (Published May 1994)

No. 25 1-Chloro-1,2,2,2-tetrafluoroethane (HCFC-124) (CAS No. 2837-89-0) (Published July 1994) (Updated by JACC No. 46)

No. 26 Linear Polydimethylsiloxanes (CAS No. 63148-62-9) (Published September 1994)

No. 27 *n*-Butyl Acrylate (CAS No. 141-32-2) (Published August 1994)

No. 28 Ethyl Acrylate (CAS No. 140-88-5) (Published September 1994)

No. 29 1,1-Dichloro-1-fluoroethane (HCFC-141b) (CAS No. 1717-00-6) (Published December 1994)

No. 30 Methyl Methacrylate (CAS No. 80-62-6) (Published February 1995)

No. 31 1,1,1,2-Tetrafluoroethane (HFC-134a) (CAS No. 811-97-2) (Published February 1995) (Updated by JACC No. 50)

No. 32 Difluoromethane (HFC-32) (CAS No. 75-10-5) (Published May 1995)

No. 33 1,1-Dichloro-2,2,2-trifluoroethane (HCFC-123) (CAS No. 306-83-2) (Published February 1996) (Updated by JACC No. 47)

No. 34 Acrylic Acid (CAS No. 79-10-7) (Published September 1995)

No. 35 Methacrylic Acid (CAS No. 79-41-4) (Published May 1996)

No. 36 *n*-Butyl Methacrylate; Isobutyl Methacrylate (CAS No. 97-88-1) (CAS No. 97-86-9) (Published December 1996)

No. 37 Methyl Acrylate (CAS No. 96-33-3) (Published September 1998)

No. 38 Monochloroacetic Acid (CAS No. 79-11-8) and its Sodium Salt (CAS No. 3926-62-3) (Published June 1999)

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No. 44 1, 1, 1,3,3-Pentafluoropropane (HFC-245fa) (Published June 2004)

No. 45 1, 1-Difluoroethane (HFC-152a) (CAS No. 75-37-6) (Published September 2004)

No. 46 1-Chloro-1,2,2,2-tetrafluoroethane (HCFC-124) CAS No. 2837-89-0 (Third Edition) (Published November 2004)

No. 47 1,1-Dichloro-2,2,2-trifluoroethane (HCFC-123) CAS No. 306-83-2 (Third Edition) (Published May 2005)

No. 48 Hexafluoropropylene (HFP) CAS No. 116-15-4 (Published September 2005)

No. 49 Vinylidene Fluoride CAS No. 75-38-7 (Published November 2005)

No. 50 1,1,1,2-Tetrafluoroethane (HFC-134a) (CAS No. 811-97-2) (Second Edition) (Published January 2006)

No. 51 Synthetic Amorphous Silica (CAS No. 7631-86-9) (Published August 2006)

No. 52 Trifluoroethane (HFC-143a) (CAS No. 420-46-2) (Published October 2006)

Special Reports

No.	Title
No. 8	HAZCHEM; A Mathematical Model for Use in Risk Assessment of Substances (Published October 1994)
No. 9	Styrene Criteria Document (Published June 1995)
No. 10	Hydrogen Peroxide OEL Criteria Document (CAS No. 7722-84-1) (Published July 1996)
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No. 12	1,3-Butadiene OEL Criteria Document (Second Edition) (CAS No. 106-99-0) (Published January 1997)
No. 13	Occupational Exposure Limits for Hydrocarbon Solvents (Published August 1997)
No. 14	*n*-Butyl Methacrylate and Isobutyl Methacrylate OEL Criteria Document (Published May 1998)
No. 15	Examination of a Proposed Skin Notation Strategy (Published September 1998)
No. 16	GREAT-ER User Manual (Published March 1999)
No. 17	Risk Assessment Report for Existing Substances Methyl *tertiary*-Butyl Ether (Published December 2003)

Documents

No.	Title
No. 32	Environmental Oestrogens: Male Reproduction and Reproductive Development (Published January 1996)
No. 33	Environmental Oestrogens: A Compendium of Test Methods (Published July 1996)
No. 34	The Challenge Posed by Endocrine-disrupting Chemicals (Published February 1996)
No. 35	Exposure Assessment in the Context of the EU Technical Guidance Documents on Risk Assessment of Substances (Published May 1997)
No. 36	Comments on OECD Draft Detailed Review Paper: Appraisal of Test Methods for Sex-Hormone Disrupting Chemicals (Published August 1997)
No. 37	EC Classification of Eye Irritancy (Published December 1997)
No. 38	Wildlife and Endocrine Disrupters: Requirements for Hazard Identification (Published January 1998)
No. 39	Screening and Testing Methods for Ecotoxicological Effects of Potential Endocrine Disrupters: Response to the EDSTAC Recommendations and a Proposed Alternative Approach (Published January 1999)
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No. 42	Genomics, Transcript Profiling, Proteomics and Metabonomics (GTPM). An Introduction (Published April 2001)
No. 43	Contact Sensitisation: Classification According to Potency. A Commentary (Published July 2003)
No. 44	Guidance for the Interpretation of Biomonitoring Data (Published November 2005)

Workshop Reports

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No. 2	Strategy Report on Challenges, Opportunities and Research needs arising from the Definition, Assessment and Management of Ecological Quality Status as required by the EU Water Framework Directive based on the workshop EQS and WFD versus PNEC and REACH - are they doing the job? 27-28 November 2003, Budapest (Published March 2004)
No. 3	Workshop on the Use of Human Data in Risk Assessment 23-24 February 2004, Cardiff (Published November 2004)
No. 4	Influence of Maternal Toxicity in Studies on Developmental Toxicity 2 March 2004, Berlin (Published October 2004)
No. 5	Workshop on Alternative Testing Approaches in Environmental Risk Assessment 7-9 July 2004, Paris (Published December 2004)
No. 6	Workshop on Chemical Pollution Respiratory Allergy and Asthma, 16-17 June 2005, Leuven (Published December 2005)
No. 7	Workshop on Testing Strategies to Establish the Safety of Nanomaterials, 7-8 November 2005, Barcelona (Published August 2006)
No. 8	Workshop on Societal Aspects of Nanotechnology, 9 November 2005, Barcelona (Published October 2006)
No. 9	Workshop on the Refinement of Mutagenicity / Genotoxicity Testing, 23-24 April 2007, Malta (Published September 2007)
No. 10	Workshop on Biodegradation and Persistence, 26-27 June 2007, Holmes Chapel (Published September 2007)