



**MIXTURE TOXICITY ASSESSMENT FOR PRECIOUS
METAL CYANIDE SUBSTANCES (Revision
following receipt of ecotoxicity test results)**

**DRAFT REPORT TO PRECIOUS METALS AND
RHENIUM CONSORTIUM FROM WCA
ENVIRONMENT LIMITED**

JUNE 2012



**wca environment limited
Brunel House
Volunteer Way
Faringdon
Oxfordshire
SN7 7YR
UK**

**Email: solutions@wca-environment.com
Web: www.wca-environment.com**

Report details

Report Title	Mixture toxicity assessment for precious metal cyanide substances (Revision following receipt of ecotoxicity test results)
Date of production	June 2012
Contract/Project Number	P0186-09/10
Client	Precious Metals and Rhenium Consortium
Client Contract Manager	Caroline Braibant
Author(s)	Adam Peters
wca Project Co-ordinator	Becky Marks
wca Project Executive	Dawn Maycock

Report Quality Check

	Printed name & Signature	Date
Document Approved by	 Adam Peters	21.06.12
Document Quality Checked by	 Dawn Maycock	15.06.12

EXECUTIVE SUMMARY

This report aims to predict the acute toxicity of gold and silver cyanide compounds based on information about the acute toxicity of the individual components (i.e. gold, silver, and cyanide). Information on the toxicity of the different components is combined with predictions of the speciation of the precious metal cyanide compounds in typical ecotoxicity test media. All chemical speciation calculations were performed using VisualMINTEQ (Version 3.0).

Precious metal cyanides are pure inorganic substances which are composed of components which are better understood than the substances themselves. The relevant components are the precious metals silver and gold, and cyanide. It has been proposed that, as an alternative to undertaking ecotoxicity testing on the substances, it may be possible to predict the toxicity of a precious metal cyanide solution from information on the toxicities of the precious metal and cyanide, and information about the chemical speciation of the precious metal cyanide substance in the test medium.

For cyanide toxicity to *Daphnia* the average (geometric mean) EC50 value of 100 $\mu\text{g l}^{-1}$ is used, this is equivalent to 3.85 μM "free cyanide". Invertebrates and fish appear to exhibit relatively similar sensitivity to cyanide, the most sensitive tested species in acute tests are a crab and a salmonid fish. *Daphnia magna* are the most acutely sensitive species to silver, the lowest 48 hour EC50 was 0.22 $\mu\text{g l}^{-1}$ as dissolved Ag (2 nM); other tests also showed similar sensitivity. The free Ag^+ ion activity at the EC50 in this test has been calculated as 1 nM. The interpretation of tests for which it was not possible to predict the free Ag^+ ion speciation an empirical correction factor was applied to the dissolved silver concentration in order to estimate the free Ag^+ ion activity at the EC50. Very limited information is currently available on the acute toxicity of gold. There is information on the toxicity of gold to fish, with a 96 hour LC50 value of 9.1 mg l^{-1} as Au (46.2 μM). New test results are now available for toxicity of gold, tested as tetrachloroauric acid, to *Daphnia magna* and algae, with EC50 values of 2.8 mg Au l^{-1} (14.16 μM) for *Daphnia* and 1.5 mg l^{-1} for algae (7.62 μM).

For potassium dicyanoargentate and silver cyanide, silver is likely to provide the dominant contribution to the overall toxicity, but cyanide may also contribute to a lesser extent. Cyanide is expected to be the only significant contributor to the ecotoxicity of potassium dicyanoaurate, based on the limited, currently available information on gold toxicity.

The EC50 for *Daphnia* is predicted to occur at a potassium dicyanoargentate concentration of 25.7 $\mu\text{g l}^{-1}$ at pH 8.4. The EC50 for fish is predicted to occur at a concentration of 3.3 mg l^{-1} and for algae is predicted as > 100 mg l^{-1} . The NOEC for STP microorganisms is predicted to be 14.2 $\mu\text{g l}^{-1}$.

The EC50 for *Daphnia* is predicted to occur at a silver cyanide concentration of 0.56 $\mu\text{g l}^{-1}$ at pH 8.4. The EC50 for fish is predicted to be 3.0 mg l^{-1} and the EC50 for algae is predicted as 80 mg l^{-1} . The NOEC for STP microorganisms is predicted to be 9.4 $\mu\text{g l}^{-1}$.

The EC50 for *Daphnia* is predicted to occur at a potassium dicyanoaurate concentration of 1.61 mg l⁻¹. The EC50 for fish is predicted to occur at 0.81 mg l⁻¹ and the EC50 for algae is predicted to be 1.21 mg l⁻¹.

Daphnia test results for potassium dicyanoargentate and potassium dicyanoaurate were used to assess the validity of the predictions. There was good agreement between the predicted and measured results for potassium dicyanoargentate. It is therefore considered that the approach for predicting toxicity is reliable for the silver cyanide substances potassium dicyanoargentate and silver cyanide.

A comparison of the measured and modelled toxicity of potassium dicyanoaurate suggests that the approach taken to predict the EC50 for this substance is unlikely to be reliable. There are several possible reasons for the poor prediction of the toxicity of gold cyanide compounds, including the oxidation state of gold following dissolution of this substance, and the ability of VisualMINTEQ to accurately predict the speciation of gold cyanide complexes in aqueous solution. Further consideration of the ability to accurately predict the speciation of potassium dicyanoaurate solutions would be necessary in order to refine the predictions and with the currently available information it may not be possible to conduct accurate predictions for this substance. Due to the uncertainties with the speciation predictions we would recommend conducting direct testing on fish and algae with potassium dicyanoaurate in order to fill these REACH endpoints, rather than attempting to refine the modelling predictions.

Following the ecotoxicity testing and mixture toxicity modelling, we would recommend that the following results are used to fill the REACH ecotoxicity endpoints for precious metal cyanide substances.

Substance	Fish EC50 (mg l ⁻¹)	Daphnia EC50 (mg l ⁻¹)	Algae EC50 (mg L ⁻¹)	Microorganisms NOEC (mg l ⁻¹)
Potassium dicyanoargentate	3.3 (predicted)	0.022 (measured)	>100 (predicted)	0.64 (measured)
Silver cyanide	3.0 (predicted)	0.00056 (predicted)	80 (predicted)	0.0094 (predicted)
Potassium dicyanoaurate	Predictions not considered suitable for use – testing proposed	0.2 (measured)	Predictions not considered suitable for use – testing proposed	60 (measured)

On the basis of the measured and predicted EC50 values for potassium dicyanoargentate and silver cyanide, these substances would be assigned a classification of Acute category 1, Chronic category 1 under the CLP regulation. This is the same as the classification that was submitted for the CLP notification for these substances. The predictions for potassium dicyanoaurate are not considered to be reliable and therefore will not be used for REACH registration or classification purposes. However, based on the measured *Daphnia* result for

this substance a classification of Acute category 1, Chronic category 1 would also apply for this substance.

CONTENTS

EXECUTIVE SUMMARY.....	i
CONTENTS	iv
TABLES	v
FIGURES.....	vi
1 INTRODUCTION	1
2 METHODS	3
2.1 Concentration Addition.....	3
2.2 Predicting Toxicity to Test Species	4
3 MIXTURE TOXICITY CALCULATIONS.....	5
3.1 Physico-chemical properties of cyanide.....	5
3.2 Acute toxicity of cyanide.....	5
3.2.1 Acute Cyanide Toxicity to <i>Daphnia</i>	6
3.2.2 Acute Cyanide Toxicity to fish.....	7
3.2.3 Acute Cyanide Toxicity to algae.....	8
3.2.4 Acute Cyanide Toxicity to microorganisms.....	8
3.3 Acute toxicity of silver	8
3.3.1 Acute silver toxicity to <i>Daphnia</i>	8
3.3.2 Acute silver toxicity to fish	9
3.3.3 Acute silver toxicity to algae.....	9
3.3.4 Acute silver toxicity to microorganisms	9
3.4 Silver cyanide speciation in aqueous media	9
3.5 Modelled Medium compositions.....	11
3.6 Toxicity of silver cyanide compounds	12
3.7 Acute Toxicity of Gold	14
3.8 Gold cyanide speciation.....	14
3.9 Toxicity of gold cyanide compounds.....	15
4 TOXICITY TESTING WITH <i>DAPHNIA</i>	17
4.1 Potassium dicyanoargentate	17
4.2 Potassium dicyanoaurate.....	18
4.3 Tetrachloroauric acid.....	19
5 TOXICITY TESTING WITH MICROORGANISMS	21
5.1 Potassium dicyanoargentate	21
6 CONCLUSIONS	22
REFERENCES	24

TABLES

Table 1.1 Cyanide content and water solubility (mg l^{-1}) of the substances considered.....	2
Table 3.1 Physico-chemical information for cyanide.....	5
Table 3.2 Dissociation constants for some metal cyanide complexes.....	6
Table 3.3 Sensitivity and variability of <i>Daphnia</i> species to free cyanide (taken from ECETOC 2007)	6
Table 3.4 Percentage distribution of principal cyanide and silver species in ecotoxicity test media for different concentrations of added potassium dicyanoargentate.	10
Table 3.5 Chemical Composition of EPA 'Moderately Hard Water' at pH 8.3.....	11
Table 3.6 Chemical Composition of OECD Synthetic Sewage Mixture	11
Table 5.1 Predicted acute EC50 values for precious metal cyanide substances.	23
Table 5.2 Predicted NOEC values for toxicity to microorganisms for precious metal cyanide substances.....	23

FIGURES

Figure 3.1	Effect of temperature on cyanide toxicity to <i>Daphnia pulex</i>	7
Figure 3.2	Speciation of silver and cyanide in ecotoxicity test media containing 1 nM AgCN	10
Figure 3.3	Predicted Toxicity Ratio as a function of the concentration of potassium dicyanoargentate added at pH 8.4 for silver, cyanide, and the total toxicity.	12
Figure 3.4	Speciation of gold and cyanide in ecotoxicity test media containing 1 nM AuCN ₃	14
Figure 3.5	Amount of each individual component added in the KAu(CN) ₂ titration.	15
Figure 4.1	Results of range finding test for potassium dicyanoargentate toxicity to <i>Daphnia</i> . The vertical red line indicates the predicted EC50.	17
Figure 4.2	Results of definitive test for potassium dicyanoargentate toxicity to <i>Daphnia</i> . The vertical red line indicates the predicted EC50.....	18
Figure 4.3	Results of range finding test for potassium dicyanoaurate toxicity to <i>Daphnia</i> . The vertical red line indicates the predicted EC50.....	18
Figure 4.4	Results of definitive test for potassium dicyanoaurate toxicity to <i>Daphnia</i> . The vertical red line indicates the predicted EC50.	19
Figure 4.5	Results of tetrachloroauric acid toxicity to <i>Daphnia</i> , with exposures expressed as concentrations of gold. The vertical red line indicates the EC50 value for fish	20

1 INTRODUCTION

This report aims to predict the acute toxicity of gold and silver cyanide compounds based on information about the acute toxicity of the individual components (i.e. gold, silver, and cyanide). Information on the toxicity of the different components is combined with predictions of the speciation of the precious metal cyanide compounds in typical ecotoxicity test media.

In order to minimise the need for animal testing, especially in cases where a large body of ecotoxicity information already exists, a calculation approach is proposed for determining the toxicity of precious metal cyanide substances using information about the toxicity of the metal ion, the toxicity of free cyanide, and the speciation of the precious metal cyanide substance in the test solution. If deemed to be appropriate this approach will avoid the need for testing the toxicity of the precious metal cyanide substances to fish. This calculation approach is considered to be appropriate for silver cyanide substances because of the extensive body of information on the acute toxicity of both silver and cyanide to aquatic organisms. Less information is available for gold, therefore the results of the direct testing with *Daphnia* will be used to determine whether the available information is sufficient to make predictions for the gold cyanide substance.

Mixture toxicity has been assessed according to the "Concentration Addition" method. This method considers the relative risk, or pressure, exerted by each potentially toxic component on the basis of the risk characterisation ratio, and the risk characterisation ratios for each of the individual components are added together to determine the overall risk. This approach has also been termed the Cumulative Criterion Unit approach in some studies (US EPA 1986; Clements et al. 2000; Hirst et al. 2002).

The concentration addition approach assumes a common mechanism of toxicity, which is unlikely to be the case for these substances. Technically a more appropriate mechanism is Independent Action, which does not assume that all the components in the mixture have the same mechanism of toxicity. However, predictions based on concentration addition are often more conservative than independent action, so using concentration addition can be considered conservative. In fact, the EU SCHER committee also proposes the use of concentration addition as a conservative means of assessing risk of mixtures in its recent opinion on mixtures¹.

Informal discussions with European regulators have suggested that the proposed approach is likely to be acceptable provided that it is validated by testing, and that this supports the approach taken.

There are three precious metal cyanide compounds requiring REACH registration, potassium dicyanoargentate, silver cyanide and potassium dicyanoaurate. All three of these substances require Annex VIII ecotoxicity endpoints to be fulfilled. The formulae, cyanide content and water solubilities of these are shown in Table 1.1.

¹ http://ec.europa.eu/health/scientific_committees/environmental_risks/docs/scher_o_150.pdf

Table 1.1 Cyanide content and water solubility (mg l⁻¹) of the substances considered

Substance Name	CAS RN	Formula	% CN	Water Sol
Potassium dicyanoargentate	506-61-6	KAg(CN) ₂	26.1	250000
Silver cyanide	506-64-9	AgCN	19.4	0.0011
Potassium dicyanoaurate	13967-50-5	KAu(CN) ₂	18.1	143000

Ecotoxicity may be affected by both the toxicity and availability of the cyanide component, as well as the toxicity of the metal component, and read-across of toxicity data from the metal, and cyanide, will need to take account of the fact that there is more than one potentially toxic component.

The testing strategy separates the gold and silver cyanide compounds because silver is highly toxic to some aquatic species, and it may be inappropriate to assume a similar level of toxicity for gold. The available data for gold for fish, *Daphnia* and algae suggests a relatively low level of toxicity, compared to silver.

The present assessment of the toxicity of precious metal cyanide substances does not take into account any differences in the environmental fate and behaviour of the metal and cyanide species which are expected to contribute to the observed toxicity. This may be an important factor in risk assessment if it is necessary to assess the potential effects of releases of these substances to the environment, especially if they are subject to wastewater treatment or are discharged via municipal sewage treatment plants. The approach taken for predicting the results of ecotoxicity tests considers the speciation of the precious metal cyanide substance in the test media, to provide a predicted EC50 which is expressed in terms of the added concentration of the substance.

For risk assessment purposes it is recommended to predict the toxicity, based on separate exposure estimates for both the metal and cyanide components, for the speciation in the receiving water. By taking this approach it will be possible to take account of the different behaviour of the metal and cyanide components during wastewater treatment processes (including treatment at a municipal STP).

2 METHODS

Precious metal cyanides are pure inorganic substances which are composed of components which are better understood than the substances themselves. The relevant components are the precious metals silver and gold, and cyanide. It has been proposed that, as an alternative to undertaking ecotoxicity testing on the substances, it may be possible to predict the toxicity of a precious metal cyanide solution from information on the toxicities of the precious metal and cyanide, and information about the chemical speciation of the precious metal cyanide substance in the test medium.

There are essentially two different approaches towards assessing the toxicity of mixtures (DG Environment 2009), and both approaches will be employed in this study to fulfil the ecotoxicity testing requirements under REACH for precious metal cyanide substances. These are whole mixture approaches, in which the toxicity of the mixture is assessed directly. Standardised ecotoxicity test methods are appropriate for such assessments. This is an empirical type of assessment and cannot indicate the chemical cause of the observed effects. The alternative approach is to assess the toxicity on a component basis, based on analysis or prediction of the concentrations of the various toxicants, and taking account of all of the relevant components in expressing the toxicity. This is a predictive type of assessment which assigns the toxicity to a number of individual components.

The direct testing approach will be used to validate the results of predictions of the toxicity of the precious metal cyanide compounds based on chemical speciation predictions of their forms in the exposure medium and an assessment of the overall toxicity due to the metal and the cyanide. All chemical speciation calculations were performed using VisualMINTEQ (Version 3.0, Gustafsson 2011).

It has been assumed that there are no synergistic or antagonistic interactions between the trace metals and cyanide which affect their toxicity. Both the concentration addition and independent action models assume that there is no interaction between the components of the mixture (resulting in more or less toxicity than predicted). In practice this may not be the case. The validation of this modelling, with ecotoxicity testing, will go some way to address this. SCHER are not convinced of the relevance of antagonism and synergism at low doses¹.

2.1 Concentration Addition

The prediction of the overall toxicity of a number of separate components can be made following the concentration addition theory. Essentially concentration addition suggests that the overall toxicity can be estimated as the sum of the individual toxicities. The toxicity is identified as the ratio of the exposure concentration relative to a threshold. In the case of acute tests the threshold may be the EC50 for the organism in question, which has been established from separate testing. This approach has also been termed the Cumulative Criterion Unit (CCU) approach in some studies (US EPA 1986; Clements et al. 2000; Hirst et al. 2002).

Essentially, a metric which is comparable to a risk characterisation ratio is calculated for each of the components, and the risk characterisation ratios are summed to calculate the overall toxicity, and also the concentration at which 50% effects would occur. The predicted response (i.e. a 50% effect in tests) is expected to occur at the concentration where the sum of the individual ratios is equal to 1.

2.2 Predicting Toxicity to Test Species

The prediction of the overall toxicity which is likely to be observed from the mixture of metal and cyanide must be performed for both fish and algal species in order to meet the information requirements for the precious metal cyanide substance registrations under REACH. For silver cyanide predictions are also required for toxicity to STP microorganisms.

Where possible these predictions are made for a single standard test species, using ecotoxicity data for both the metal and free cyanide on the same species. In some cases the standard test species, or those species for which toxicity data are available for both toxicants, are not the most sensitive species to the toxicants of interest. A worst case indication of the toxicity of the metal cyanide compounds can be made by applying the most sensitive result for each toxicant to each trophic level.

3 MIXTURE TOXICITY CALCULATIONS

3.1 Physico-chemical properties of cyanide

The physico-chemical properties of cyanide are presented in Table 3.1.

Table 3.1 Physico-chemical information for cyanide

Endpoint	Value	Reference
Water solubility	1 kg l ⁻¹	ECETOC 2007
Vapour pressure	82.7 kPa	ECETOC 2007
Henry's law constant	1.33 x 10 ⁻⁴ atm m ³ mol ⁻¹	ECETOC 2007
pKa	9.36	ECETOC 2007

3.2 Acute toxicity of cyanide

Toxicity tests with cyanide have been reviewed on a number of occasions (USEPA 1984, Environment Agency 2007, ECETOC 2007) and ecotoxicity information is generally expressed as free cyanide (CN⁻). *Daphnia pulex* was found to be the most sensitive invertebrate to cyanide, although *Daphnia magna* was only slightly less sensitive. The minimum *Daphnia pulex* EC50 was 1 µg (CN) l⁻¹ and the geometric mean EC50 was 100 µg (CN) l⁻¹ as free cyanide; the most sensitive results were obtained at elevated temperatures. Rainbow trout (*Oncorhynchus mykiss*) was the most sensitive fish species, with a minimum LC50 of 7 µg (CN) l⁻¹ and a geometric mean LC50 of 57 µg (CN) l⁻¹ as free cyanide. The geometric mean EC50 values for the most sensitive fish and invertebrate species to cyanide are within a factor of 2, suggesting that there is relatively little difference in the sensitivity of these organisms to cyanide.

There is a limited amount of information on the toxicity of cyanide to algal species, and the tests are non-standard. The lowest EC50 value was 45 µg (CN) l⁻¹, as free cyanide, for *Chlorococcales* spp. in a 24 hour test.

Dissociation constants for the relevant precious metal cyanide complexes are shown in Table 3.2, along with the dissociation constants for a number of other metal cyanide complexes (ECETOC 2007). The dissociation constant effectively describes the stability of the metal cyanide complex, with low values indicating very high stability (e.g. Fe(CN)₆³⁻), and higher values indicating lower stability of the metal cyanide complex.

For *Daphnia* toxicity both the lowest ($1 \mu\text{g (CN)}^{-1}$) and average ($100 \mu\text{g (CN)}^{-1}$, geometric mean) EC50 values are considered, these are equivalent to $0.0385 \mu\text{M}$ and $3.85 \mu\text{M}$ respectively. As these effect levels are based on measured concentrations of free cyanide it is not necessary to consider the speciation of cyanide in the test media. The observed variation in sensitivity of *Daphnia* is discussed in more detail in Section 3.2.1.

Table 3.2 Dissociation constants for some metal cyanide complexes

Complex	Dissociation constant
Au(CN)_2^-	38.3
Cd(CN)_4^{3-}	16.8
Ag(CN)_2^-	-18.7
Fe(CN)_6^{4-}	-47.0
Fe(CN)_6^{3-}	-52.0

The “free cyanide” is considered to be the toxic component of cyanide solutions, and is essentially the sum of HCN and CN^- concentrations. Other cyanide species are assumed not to be toxic.

3.2.1 Acute Cyanide Toxicity to *Daphnia*

Very large variability in the results of tests on Daphnids has been noted for cyanide (ECETOC 2007), and the reasons for this variability are considered in greater detail here.

Table 3.3 Sensitivity and variability of *Daphnia* species to free cyanide (taken from ECETOC 2007)

Species	Number	EC50 values, $\mu\text{g CN}^{-1}$			
		Minimum	Geo Mean	Maximum	Range *
<i>Daphnia pulex</i>	11	1	100	420	420
<i>Daphnia magna</i>	6	40	174	1900	47.5
<i>Daphnia spp.</i>	3	80	132	173	2.16

* Ratio between maximum and minimum.

The lowest EC50 values in the ecotoxicity database (1 and $3 \mu\text{g (CN)}^{-1}$) were both derived from tests on *Daphnia pulex* (Cairns et al. 1978), all of the other EC50 values for this species were above $40 \mu\text{g (CN)}^{-1}$. Cairns et al. (1978) investigated the effect of temperature on sensitivity to cyanide toxicity for several species. At 20°C an EC50 of $110 \mu\text{g (CN)}^{-1}$ was reported. At higher temperatures Cairns et al. (1978) noted *Daphnia* were more sensitive. Results from another test conducted above 20°C also showed higher sensitivity for *Daphnia*, with an EC50 for *Daphnia pulex* of $39.8 \mu\text{g (CN)}^{-1}$ (Monsanto 1981); this test also reported a NOEC of $23.3 \mu\text{g (CN)}^{-1}$.

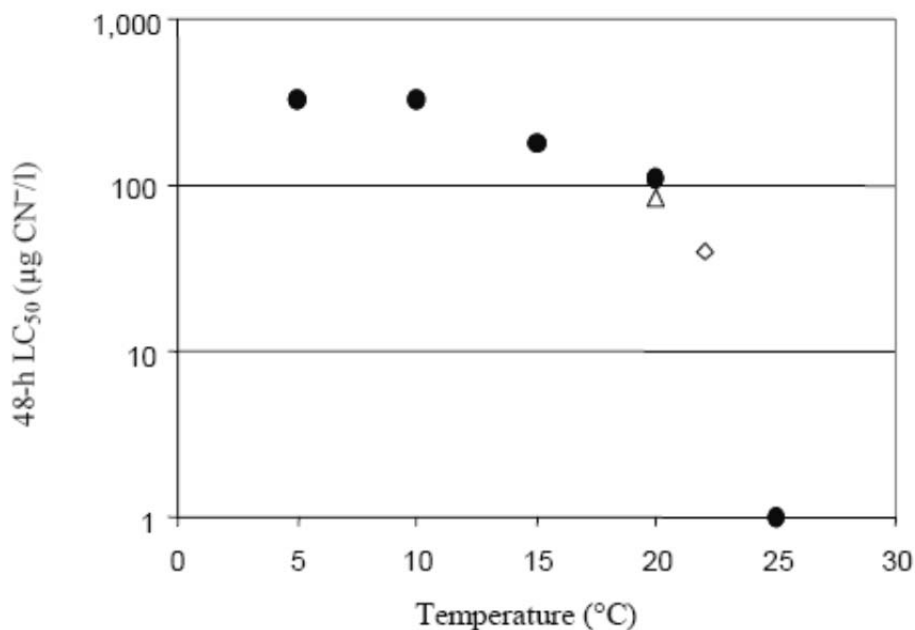


Figure 3.1 Effect of temperature on cyanide toxicity to *Daphnia pulex*.

Data from Cairns et al. 1978 (closed circles), Lee 1976* (open triangle), and Monsanto 1981 (open diamond), from ECETOC 2007.

*water temperature was not available and the standard of 20°C was assumed

The sensitivity of daphnids to temperature has been studied on a number of occasions. Macissac et al. (1985) noted that *Daphnia pulex* exhibited the greatest sensitivity to elevated temperatures. Studies of the sensitivity of *Daphnia magna* to cadmium (Heugens et al. 2003) have also shown an increased sensitivity at elevated temperatures. This study also noted particularly high sensitivity at temperatures above 20°C, which is consistent with the findings of Cairns et al. (1978) although for a different species. These studies suggest that *Daphnia* test results are influenced by temperature, with the more sensitive results observed at high temperatures.

As a result of the increased sensitivity of *Daphnia* to cyanide at elevated temperatures the average (geometric mean of all results) EC50 value of 100 µg (CN⁻) l⁻¹ (3.85 µM) is used for predictions of the toxicity of precious metal cyanides to *Daphnia* species. Whilst the results obtained at elevated temperatures were not excluded from the analysis the geometric mean is considered to be a fair reflection of the sensitivity of daphnids at standard test temperatures (typically 20°C).

3.2.2 Acute Cyanide Toxicity to fish

Numerous studies have been conducted on the acute toxicity of cyanide to fish (ECETOC 2007), and data are available for 19 species. The most sensitive species tested was *Oncorhynchus mykiss*, with acute LC50 values ranging from 27 to 97 µg (CN⁻) l⁻¹. The geometric mean of acute LC50 values for *Oncorhynchus mykiss* is 57 µg (CN⁻) l⁻¹. Data are also available for the toxicity of cyanide to *Pimephales promelas*, which was slightly less sensitive than *Oncorhynchus mykiss*. Thirty-five tests on this species were reported, with LC50 values ranging from 79 to 339 µg (CN⁻) l⁻¹, and a geometric mean of 137 µg (CN⁻) l⁻¹.

3.2.3 Acute Cyanide Toxicity to algae

Relatively few tests of the toxicity of cyanide to algae are available (ECETOC 2007), and in the majority of cases have not been performed in accordance with current standard testing guidelines. As a result of this there is considerable variation in the duration of the tests reported, between 24 hours and 32 days, and no data are available for the common test species *Pseudokirchneriella subcapitata*. The most sensitive result reported is for a 24 hour test with a *Chlorococcales* species which resulted in an EC50 value of 45 µg (CN⁻) l⁻¹. Similar sensitivity has also been reported for the marine species *Nitzschia closterium* in a 72 hour test (EC50 57 µg (CN⁻) l⁻¹). Tests on three other species indicated low sensitivity to free cyanide.

3.2.4 Acute Cyanide Toxicity to microorganisms

A number of tests are available for the toxicity of cyanide to microorganisms, including tests with bacteria, protozoa and activated sludge. The available results are from a number of different test types and durations. For the modelling approach, results should be taken from similar test types so that the results are comparable. Results from studies using activated sludge have therefore been selected for the toxicity predictions. The most sensitive result is from a standard OECD 209 activated sludge respiration inhibition study, with an EC20 of 0.08 mg (CN⁻) L⁻¹ based on respiratory inhibition (ECETOC 2007). This value is divided by two in order to determine the NOEC. A NOEC of 0.04 mg (CN⁻) L⁻¹ is therefore used for toxicity predictions.

3.3 Acute toxicity of silver

Toxicity tests with silver have been reviewed on a number of occasions (USEPA 1980; USEPA 1987; Environment Agency 2010) and ecotoxicity information is generally expressed as dissolved silver. Whilst there is evidence to suggest that silver toxicity may be affected by water chemistry factors such as hardness, chloride and DOC, this is often not taken into account in establishing environmental quality standards. The initial USEPA standard for silver was related to hardness, but a later update concluded that there were insufficient data at elevated hardness to allow such a criterion, due to poor agreement between different data at relatively high hardness.

The free silver ion (Ag⁺) is assumed to be the dominant toxic silver species. Some studies on other metals have suggested that some other simple inorganic complexes can also be toxic, such as hydroxides and carbonates. Effect concentrations for the critical *Daphnia* study (Bowles et al. 2002; Bianchini et al. 2002a) have been recalculated to express exposures in terms of the free ion activity of silver, and also as the sum of the activities of all inorganic silver species. The free Ag⁺ ion activity at the EC50 is 1.0 nM (equivalent to approximately 0.1 µg l⁻¹) and the activity of all inorganic silver species at the EC50 is 2.2 nM (equivalent to approximately 0.24 µg l⁻¹). EC50 values based on free ion activities were calculated using TRAP (Version 1.20, USEPA 2008).

3.3.1 Acute silver toxicity to *Daphnia*

Daphnia magna were the most acutely sensitive species to silver toxicity, although *Ceriodaphnia dubia* showed only slightly lower sensitivity. The lowest 48 hour EC50 was $0.22 \mu\text{g l}^{-1}$ in a Klimisch 1 study by Bianchini et al. (2002a), using the test system described by Bowles et al. (2002), although numerous other tests showed similar sensitivity (e.g. Bianchini et al. 2002b, Klimisch 2).

3.3.2 Acute silver toxicity to fish

The most sensitive fish species in acute toxicity tests with silver was *Pimephales promelas*, with a 96 hour EC50 value of $1.2 \mu\text{g l}^{-1}$ dissolved silver (Bielmyer et al 2007, Klimisch 2). Data are also available for the toxicity of silver to *Oncorhynchus mykiss*, which was found to be slightly less sensitive with an LC50 value of $3.3 \mu\text{g l}^{-1}$ dissolved silver (Morgan et al. 2004, Klimisch 2). Details of the water chemistry of these tests were not available to enable calculation of the test endpoints on a free Ag^+ basis. In the absence of this information the fraction of dissolved silver which was present as free Ag^+ in the critical *Daphnia* test (0.44) was also assumed for these tests.

3.3.3 Acute silver toxicity to algae

A 72 hour EC50 value of $9.7 \mu\text{g l}^{-1}$ dissolved silver was reported for a test on *Pseudokirchneriella subcapitata* which was conducted by NIVA. Other studies have reported lower sensitivity for two *Chlorella* species (10 to $100 \mu\text{g l}^{-1}$), in tests of unknown duration, and for the saltwater alga *Skeletonema costatum* ($130 \mu\text{g l}^{-1}$) (US EPA 1980).

3.3.4 Acute silver toxicity to microorganisms

A range of results are available from studies assessing the toxicity to microorganisms from silver. A number of different study types and exposure periods were used. For consistency, the lowest toxicity result related to STP functioning is used in the modelling approach. Choi et al. 2008 studied the inhibition of autotrophic nitrification using a population of nitrifying bacteria initially obtained from a municipal STP. The LOEC was between 10 and 20% inhibition and therefore the LOEC value has been divided by two to estimate the NOEC. The NOEC based on inhibition of nitrification is $0.025 \text{ mg Ag l}^{-1}$. Details of the water chemistry of these tests were not available to enable calculation of the test endpoints on a free Ag^+ basis. In the absence of this information the fraction of dissolved silver which was present as free Ag^+ in the critical *Daphnia* test (0.44) was also assumed for these tests.

3.4 Silver cyanide speciation in aqueous media

Cyanide may be an important contributor to the toxicity of silver cyanide as HCN is expected to be present at levels around 10 times higher than either CN^- or Ag^+ under the conditions present in typical ecotoxicity tests. Cyanide speciation is dominated by HCN over the pH range 7.0 to 8.6 (Figure 3.2), although the concentrations decline rapidly above pH 8.

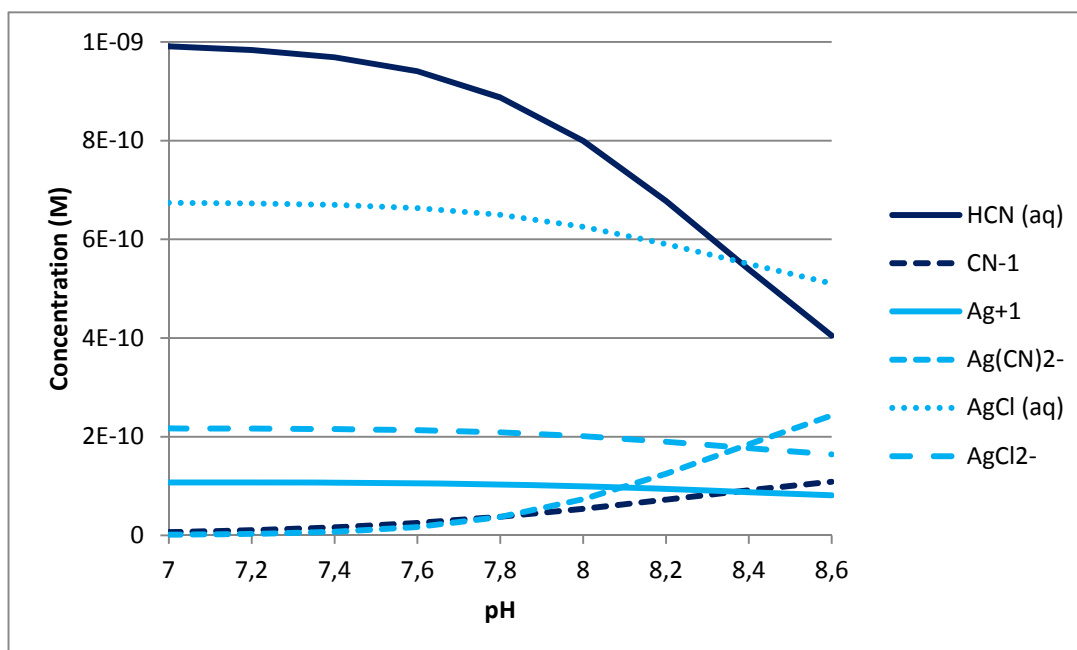


Figure 3.2 Speciation of silver and cyanide in ecotoxicity test media containing 1 nM AgCN

The dominant silver species is predicted to be the neutral silver chloride complex, which would not be expected to contribute directly to toxicity according to the “Biotic Ligand” theory. The solution speciation of both Ag and CN is also predicted to be dominated by the same species (HCN and AgCl) in the presence of $0.1 \mu\text{M}$ Fe(III). Concentrations of $\text{Fe}(\text{CN})_6^{3-}$ (the hexacyanoferrate ion), and related complexes, are predicted to be extremely low ($< 1 \times 10^{-30}$ M). Increasing levels of added potassium dicyanoargentate cause a shift in the speciation towards the formation of the $\text{Ag}(\text{CN})_2^-$ ion, which dominates the speciation of both silver and cyanide as the concentrations increase. The proportional distribution of some key silver and cyanide species, at different concentrations of potassium dicyanoargentate, are shown in Table 3.4.

Table 3.4 Percentage distribution of principal cyanide and silver species in ecotoxicity test media for different concentrations of added potassium dicyanoargentate.

	Species	$3 \mu\text{g l}^{-1}$ $\text{KAg}(\text{CN})_2$ added	$10 \mu\text{g l}^{-1}$ $\text{KAg}(\text{CN})_2$ added	$21 \mu\text{g l}^{-1}$ $\text{KAg}(\text{CN})_2$ added
Cyanide	HCN^0 (aq)	24.1	11.3	7.1
	$\text{Ag}(\text{CN})_2^-$	74.4	88.0	92.5
Silver	Ag^+	11.2	5.2	3.3
	AgCl^0 (aq)	13.7	6.4	4.0
	$\text{Ag}(\text{CN})_2^-$	74.4	88.0	92.5

3.5 Modelled Medium compositions

Two different mediums were used to model toxicity to fish, algae, and microorganisms in VISUAL MINTEQ. For the assessment of silver and cyanide toxicity to fish and algae during modelling, the EPA 'Moderately Hard Water' medium was used as the dilution water (Table 3.5).

Table 3.5 Chemical Composition of EPA 'Moderately Hard Water' at pH 8.3

Compound	Concentration (mg l ⁻¹)	Concentration (g l ⁻¹)	Concentration (Mol)	Component	Concentration (Mol)
NaHCO ₃	192	0.192	0.0023	Na ⁺	0.00063
CaSO ₄	120	0.12	0.0009	H ⁺	2.7E-05
MgSO ₄	245	0.245	0.0020	CO ₃ ⁻	0.0016
KCl	8	0.008	0.0001	Ca ²⁺	0.00026
				SO ₄ ²⁻ (as CaSO ₄)	0.00062
				SO ₄ ²⁻ (as MgSO ₄)	0.0016
				SO ₄ ²⁻ (total)	0.0022
				Mg ²⁺	0.00041
				K ⁺	5.6E-05
				Cl ⁻	5.1E-05

For modelling the effect of acute cyanide toxicity to microorganisms in Visual MINTEQ, the OECD guideline synthetic sewage used in the Potassium dicyanoargentate, Activated Sludge Respiration Inhibition Test (Report BR0626/B, Brixham Environmental Laboratory, 2012e) was modelled. The composition of the ASRIT medium is detailed in Table 3.6; the same medium was also used to assess the acute silver toxicity to microorganisms.

Table 3.6 Chemical Composition of OECD Synthetic Sewage Mixture

Compound	Concentration (g l ⁻¹)	Concentration (Mol)	Component	Concentration (Mol)
NaCl	0.7	0.012	Na ⁺	0.0047
CaCl ₂	0.4	0.0036	Cl ⁻ (as NaCl)	0.0073
MgSO ₄	0.2	0.0017	Cl ⁻ (as CaCl ₂)	0.0023
K ₂ HPO ₄	2.8	0.016	Cl ⁻ (Total)	0.0096
UREA; CH ₄ N ₂ O	3	0.050	Ca ²⁺	0.0013
Peptone	16	N.D.	Mg ²⁺	0.00034
Meat Extract	11	N.D.	SO ₄ ²⁻	0.0013
			K ⁺	0.0072
			H ⁺	9.3E-05

The UREA, peptone and meat extract compound could not be modelled in Visual MINTEQ, as these complex organic compounds are not included in the models suite of organic compounds. However, failure to take account of any toxicity or availability modifying effect of these components in the results produced during the modelling, results in conservative predictions for the toxicity of the precious metal cyanide substances, due to a simplified system being modelled.

In both scenarios, a multi-problem sweep titration was modelled for the addition of silver, cyanide and potassium. The volume of solution to be titrated was defined as 200 arbitrary volume units, with the titrant volume added at each step defined as 0.0001. The concentration of silver, cyanide and potassium added at each step is defined as 0.001, 0.002 and 0.001 Mol l⁻¹, respectively.

3.6 Toxicity of silver cyanide compounds

The increase in toxicity due to both the individual components and the overall predicted toxicity are shown, in Figure 3.3, as a function of the volume of potassium dicyanoargentate titrant added.

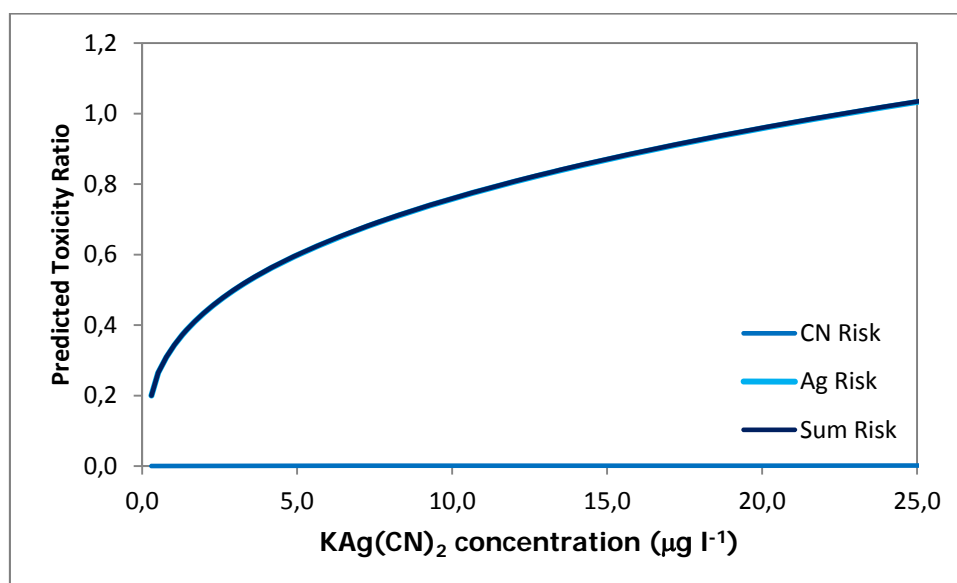


Figure 3.3 Predicted Toxicity Ratio as a function of the concentration of potassium dicyanoargentate added at pH 8.4 for silver, cyanide, and the total toxicity.

Simulated titrations of a natural water with potassium dicyanoargentate at pH 7.5 indicate that silver is likely to provide the dominant contribution to the overall toxicity, and that cyanide does not contribute significantly to the expected toxicity (except if the lowest EC50 for *Daphnia* is taken into account).

Exposures to individual components and the overall mixtures are expressed as a Predicted Toxicity Ratio (PTR), which is the predicted concentration of the component divided by the concentration required to cause a 50% effect in the test subjects. The concentrations of the separate toxicants cannot be added together to indicate the potential toxicity of the mixture,

but their Predicted Toxicity Ratios can be added to indicate the overall potential for toxicity. A Predicted Toxicity Ratio of 1 is calculated for each individual component when the concentration is equal to the EC50. It is assumed that when both toxicants act together they cause the same level of effect at the same Predicted Toxicity Ratio.

The EC50 for *Daphnia* is predicted to occur (Σ PTR of individual elements = 1) at an added potassium dicyanoargentate concentration of 0.13 μM (25.7 $\mu\text{g l}^{-1}$) at pH 8.4 (the pH of the ecotoxicity tests) and on the basis of the EC50 values for *Daphnia* of 1 nM free Ag^+ and 3.85 μM free CN. This assumes that little or no DOC is present in the system. These tests could be very sensitive to variation in water quality conditions (particularly DOC and sulphide) due to the possible reduction in silver bioavailability attributable to increasing concentrations of DOC or sulphide. The concentrations of both DOC and sulphide in an ecotoxicity test should, however, generally be very low.

The toxicity of silver cyanide (AgCN) to *Daphnia* has also been estimated following the same principles as used for potassium dicyanoargentate. As was observed for potassium dicyanoargentate, silver is predicted to be the principal toxicant, with cyanide not expected to contribute significantly to the overall toxicity at the EC50 (less than 1% of toxicity due to CN^-). The EC50 for silver cyanide is predicted to occur at an added concentration of 4.2 nM (0.56 $\mu\text{g l}^{-1}$).

The EC50 for potassium dicyanoargentate toxicity to *Oncorhynchus mykiss* is predicted to be 16.1 mg l^{-1} . The EC50 for potassium dicyanoargentate toxicity to *Pimephales promelas* is predicted to be 3.3 mg l^{-1} . The difference in sensitivities between these species is predominantly due to their different sensitivity to silver, which is predicted to be the main toxicant in tests with potassium dicyanoargentate. The EC50 for potassium dicyanoargentate toxicity to algal species is predicted to be $>100 \text{ mg l}^{-1}$. The NOEC for potassium dicyanoargentate toxicity to STP microorganisms is conservatively predicted to be 0.0142 mg l^{-1} .

The EC50 for silver cyanide toxicity to *Oncorhynchus mykiss* is predicted to be 8.4 mg l^{-1} . The EC50 for silver cyanide toxicity to *Pimephales promelas* is predicted to be 3.0 mg l^{-1} . The difference in sensitivities between these species is predominantly due to their different sensitivity to silver, which is predicted to be the main toxicant in tests with silver cyanide. The EC50 for silver cyanide toxicity to algal species is predicted to be 80 mg l^{-1} . The NOEC for silver cyanide toxicity to STP microorganisms is conservatively predicted to be 0.0094 mg l^{-1} .

Much higher effect concentrations are predicted for the fish and algae than for *Daphnia*. This is due to the greater sensitivity of *Daphnia* to acute silver toxicity, and the importance of silver cyanide complexes in the speciation of both silver and cyanide as concentrations of added potassium dicyanoargentate increase. When Ag^+ and CN^- concentrations are both low additions of silver cyanide substances predominantly contribute to increasing the activities of the free ions. As higher concentrations are reached further additions of the silver cyanide substances result in the formation of greater quantities of the silver cyanide complex ($\text{Ag}(\text{CN})_2^-$), and increases in the activities of the free ions are limited. This effect can be seen in Figure 3.3 where small additions of potassium dicyanoargentate initially cause a large

increase in the predicted toxicity ratio, where as at higher concentrations much larger additions are required to achieve the same increase in the predicted toxicity ratio.

3.7 Acute Toxicity of Gold

Limited information is currently available on the acute toxicity of gold. Transformation/Dissolution (T/D) test results are available for gold powder that determine dissolution to be lower than the limit of detection ($0.3 \mu\text{g l}^{-1}$) (ECTX 2012). Testing has been conducted on tetrachloroauric acid for REACH registration. The likely oxidation states of any dissolved gold are Au^+ and Au^{3+} . Colloidal Au^0 could also be a possibility, although the release of a metal from the solid to solution usually implies some kind of corrosion reaction, with a change in oxidation state. By analogy with Ag, Au^+ might be expected to be the most likely oxidation state of any dissolved Au, although Cu^+ , which is in the same group as both Ag and Au, is generally unstable in aqueous solutions. The limited available water solubility information suggests that Au^{3+} is probably the most relevant species in natural waters. There is information on the toxicity of gold to fish, with a 96 hour LC_{50} value of 9.1 mg l^{-1} as Au ($46.2 \mu\text{M}$) (Buhl and Hamilton 1991, Klimisch 2).

The acute toxicity of gold (as tetrachloroauric acid) to *Daphnia magna* and *Pseudokirchneriella subcapitata* has been assessed separately, and this information is also used to inform the ecotoxicity modelling for gold cyanide substances. A 72 hour E_bC_{50} value of 1.5 mg l^{-1} dissolved gold ($7.62 \mu\text{M}$) has been reported for *Pseudokirchneriella subcapitata* (Brixham Environmental Laboratory 2012a), and a 48 hour EC_{50} of 2.8 mg l^{-1} dissolved gold ($14.16 \mu\text{M}$) has been reported for *Daphnia magna* (Brixham Environmental Laboratory 2012b).

3.8 Gold cyanide speciation

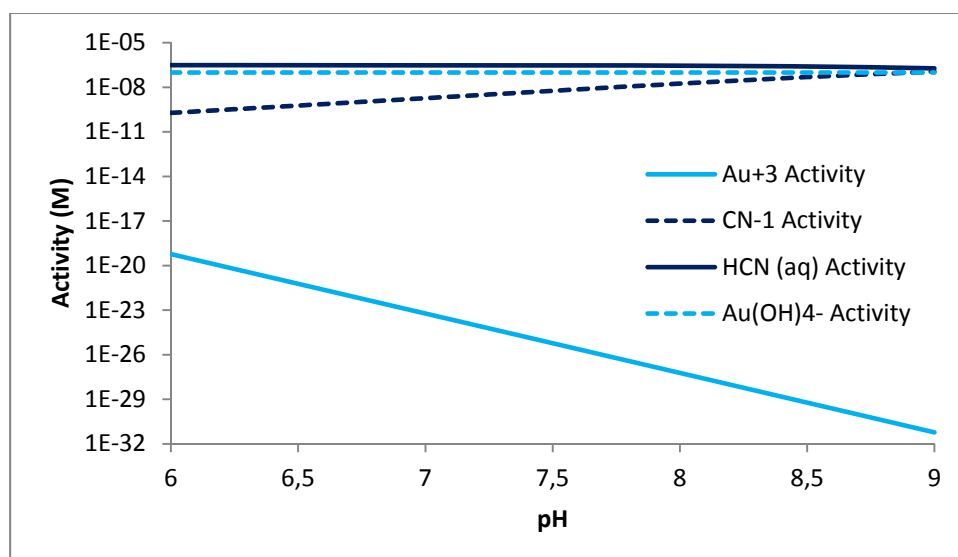


Figure 3.4 Speciation of gold and cyanide in ecotoxicity test media containing 1 nM AuCN_3

If Au(III) is the dominant form of gold then $\text{Au}(\text{OH})_4^-$ is predicted to be the dominant gold species at pH 7.5, and HCN the dominant form of cyanide (see Figure 3.4). Free Au^{3+} concentrations decline with increasing pH. At pH 7.5 HCN is likely to be the key toxicant, with levels of CN^- about 2% those of HCN, and Au^{3+} concentrations much lower.

The gold cyanide compound is potassium dicyanoaurate, in which gold is in the +1 valence state. Common chemical speciation models only include stability constants for the Au(III) valence state and it is not clear whether or not predictions made on the basis of this species will be directly relevant to solutions of potassium dicyanoaurate. Alternative speciation models, or the modification of existing models could be investigated for this purpose, although analytical confirmation of the oxidation state of any gold resulting from the dissolution of this substance may be problematic, and the only oxidation state of gold which is considered to be in a testable form is Au(III). The usefulness of such approaches may therefore be limited at present, as there are other unknown factors including the oxidation state of gold in the solution and the toxicity of the Au(I) ion.

3.9 Toxicity of gold cyanide compounds

Simulated titrations of a natural water with potassium dicyanoaurate indicate that free cyanide concentrations would be higher than free gold concentrations, and this situation is maintained as the dose of gold cyanide increases. Cyanide is expected to be the only significant contributor to the ecotoxicity of gold cyanide, based on the limited information on gold toxicity.

Toxicity is predicted for an added concentration of approximately $5.60 \mu\text{M}$ (1.61 mg l^{-1}) potassium dicyanoaurate on the basis of the EC50 for *Daphnia* of $3.85 \mu\text{M CN}^-$ ($100 \mu\text{g l}^{-1} \text{CN}^-$) and $10.7 \mu\text{M}$ (1.5 mg l^{-1}) Au with the basis for these predictions being the calculated speciation of $\text{KAu}(\text{CN})_2$. A graphical presentation of the $\text{KAu}(\text{CN})_2$ titration is presented below (Figure 3.5). Variation in pH is not expected to result in a considerable difference in *Daphnia* sensitivity to potassium dicyanoaurate on the basis of the predictions used for this report.

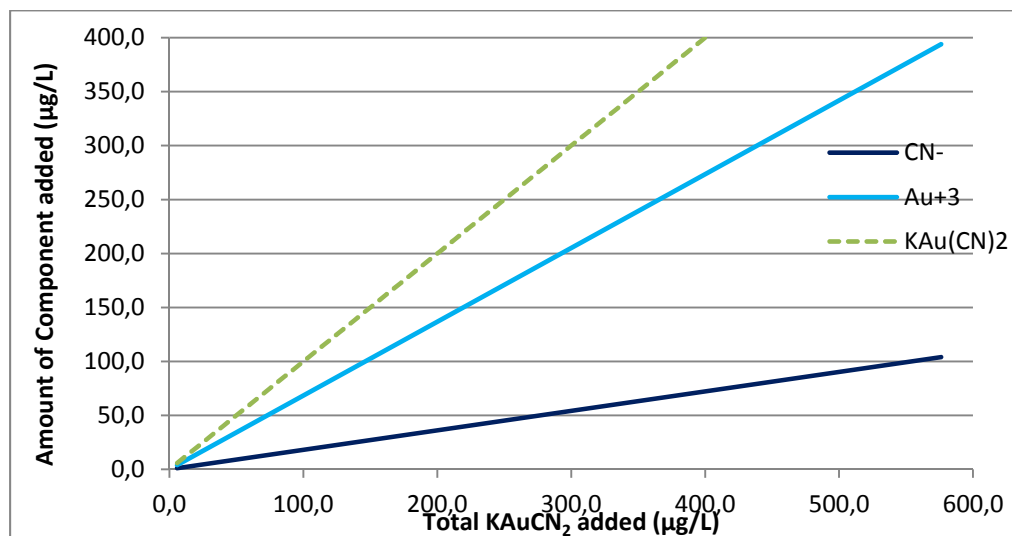


Figure 3.5 Amount of each individual component added in the $\text{KAu}(\text{CN})_2$ titration.

Both Au^{3+} and $\text{Au}(\text{OH})_4^-$ were considered as potentially toxic gold species for the estimation of EC50 values for gold cyanide substances, and were considered both independently and together (i.e. the combined effect of Au^{3+} and $\text{Au}(\text{OH})_4^-$). This had very little influence of the predicted level of the EC50 due to the much higher toxicity of cyanide than gold to *Daphnia*. The final predictions of the toxicity of potassium dicyanoaurate assume that both Au^{3+} and $\text{Au}(\text{OH})_4^-$ contribute to the toxicity of gold.

The EC50 for potassium dicyanoaurate toxicity to *Oncorhynchus mykiss* is predicted to be 0.81 mg l^{-1} . This is based on the EC50 values of $57 \text{ } \mu\text{g (CN}^{-1}) \text{ l}^{-1}$ and 9.1 mg Au l^{-1} . The EC50 for potassium dicyanoaurate toxicity to algal species is predicted to be 1.21 mg l^{-1} .

4 TOXICITY TESTING WITH *DAPHNIA*

4.1 Potassium dicyanoargentate

Range finding tests were conducted to assess the toxicity of potassium dicyanoargentate to *Daphnia magna* to identify the range within which toxicity would be observed. The results of the study are shown in Figure 4.1, along with the predicted level of the EC50. The predictions (vertical red line) are in good agreement with the initial ecotoxicity data.

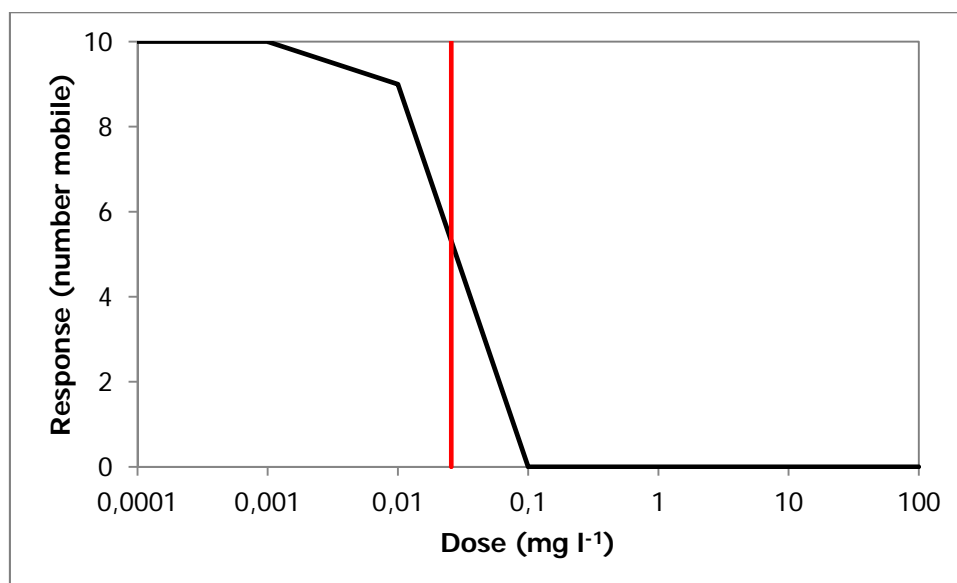


Figure 4.1 Results of range finding test for potassium dicyanoargentate toxicity to *Daphnia*. The vertical red line indicates the predicted EC50.

The results of the definitive test of the acute toxicity of potassium dicyanoargentate to *Daphnia magna* are shown in Figure 4.2. The reported 48 hour EC50 values from the test were 0.033 mg l⁻¹ (95% confidence interval 0.026 to 0.043 mg l⁻¹) based on nominal concentrations, and 0.022 mg l⁻¹ (95% confidence interval 0.016 to 0.030 mg l⁻¹) based on measured concentrations (Brixham Environmental Laboratory 2012c). The predicted 40 hour EC50 value for *Daphnia magna* of 0.0257 mg l⁻¹ is in excellent agreement with the results of the ecotoxicity testing. This supports the assertion that it is possible to predict the toxicity of silver cyanides to aquatic species based on separate toxicity data for silver and cyanide to the test species.

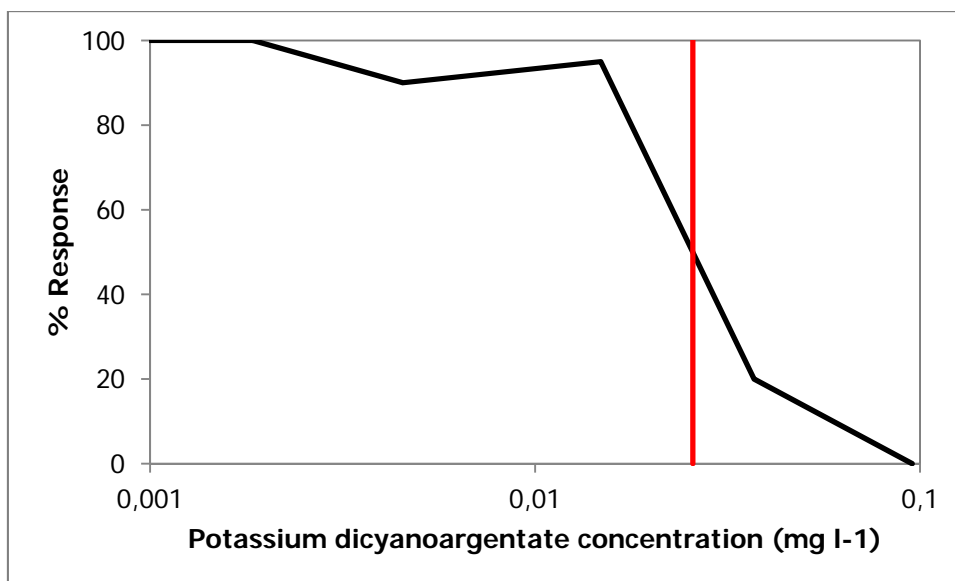


Figure 4.2 Results of definitive test for potassium dicyanoargentate toxicity to *Daphnia*. The vertical red line indicates the predicted EC50.

4.2 Potassium dicyanoaurate

Range finding tests were conducted to assess the toxicity of potassium dicyanoaurate to *Daphnia magna* to identify the range within which toxicity would be observed. The results of the study are shown in Figure 4.3, along with the predicted level of the EC50. The predictions (1.61 mg l⁻¹, vertical red line) suggest lower toxicity than was observed with the ecotoxicity test data.

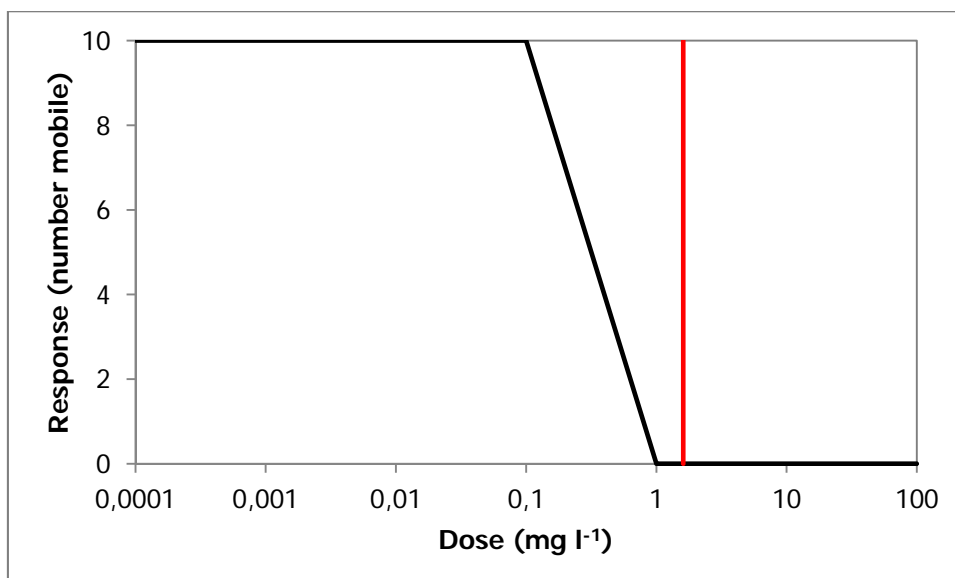


Figure 4.3 Results of range finding test for potassium dicyanoaurate toxicity to *Daphnia*. The vertical red line indicates the predicted EC50.

The results of the definitive test (Brixham Environmental Laboratory 2012d) are shown in Figure 4.4, and show a very similar situation to that seen in the range finding test.

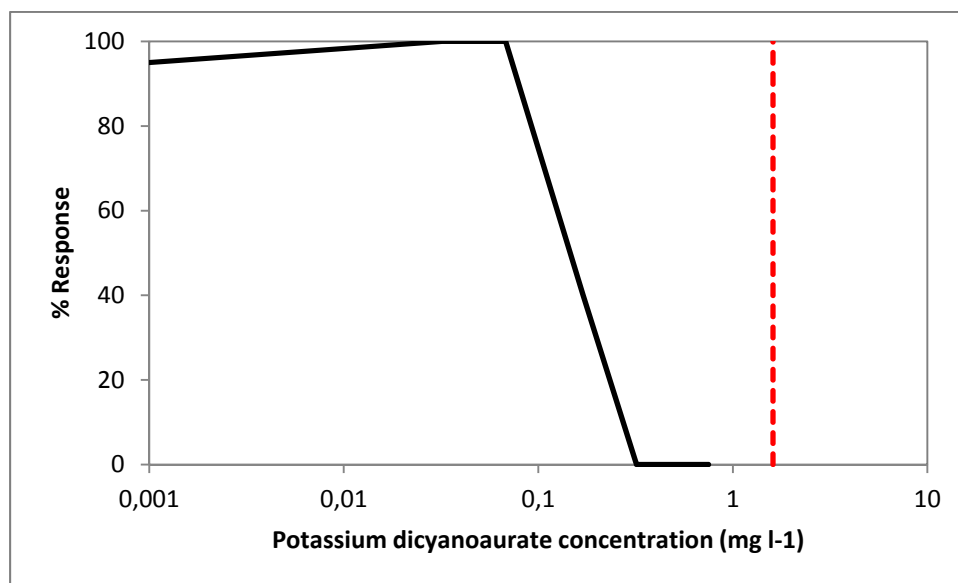


Figure 4.4 Results of definitive test for potassium dicyanoaurate toxicity to *Daphnia*. The vertical red line indicates the predicted EC50.

The results of the acute *Daphnia* test with potassium dicyanoaurate suggest that the current approach towards predicting the toxicity of this substance based on toxicity data for the constituents, and chemical speciation modelling is not suitable. This may be due to uncertainties surrounding the solution behaviour of gold, such as its oxidation state and the formation of complexes with cyanide.

As the test results indicate greater toxicity than has been predicted by the modelling calculations this is unlikely to be due to a failure to correctly model the speciation of gold and cyanide in these tests, because this would have resulted in lower activities of free gold and cyanide. The results of the tests with silver cyanide substances suggest that the principle of the modelling approach is appropriate, and that the toxicity of cyanide can be modelled effectively, at least in solutions containing silver. These considerations would tend to suggest that it is the toxicity of the gold species in solution that has been underestimated in the present study, although this may be in addition to correctly modelling the interactions between gold and cyanide in solution. This would imply that tetrachloroauric acid may not be an appropriate substance for reading across data on gold toxicity for gold cyanide substances.

4.3 Tetrachloroauric acid

Ecotoxicity tests were also conducted to assess the toxicity of tetrachloroauric acid to *Daphnia magna*, as ecotoxicity data were only previously available for fish. The results of the study are shown in Figure 4.5, along with the EC50 for fish (vertical red line). The EC50 for fish is in good agreement with the initial ecotoxicity data for *Daphnia*, supporting the initial assumption that the sensitivity of both fish and *Daphnia* to gold (III) is similar.

An EC50 for the toxicity of gold (Au[III]) to *Daphnia* is calculated as 1.72 mg l⁻¹ based on the test results for tetrachloroauric acid.

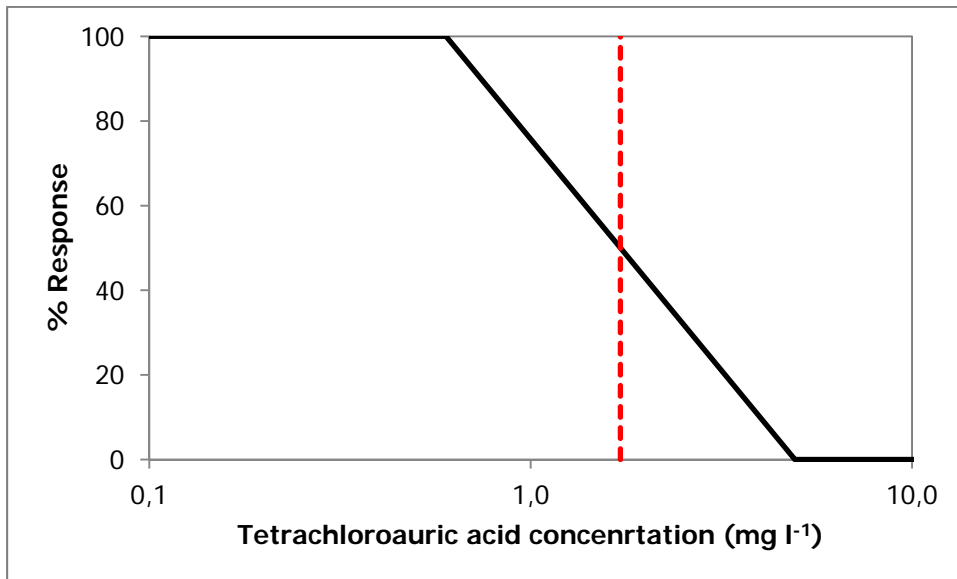


Figure 4.5 Results of tetrachloroauric acid toxicity to *Daphnia*, with exposures expressed as concentrations of gold. The vertical red line indicates the EC50 value for fish

5 TOXICITY TESTING WITH MICROORGANISMS

5.1 Potassium dicyanoargentate

The results of the definitive activated sludge respiration inhibition test were a 3-hour EC50 of 1.9 mg L⁻¹ and a 3-hour NOEC of 0.64 mg L⁻¹, based on nominal test concentrations (Brixham Environmental Laboratory 2012e). The NOEC for potassium dicyanoargentate toxicity to STP microorganisms was conservatively predicted to be 0.0142 mg l⁻¹ (14.2 µg l⁻¹). There is a margin of safety of a factor of 45 between the predicted NOEC and the measured 3-hour NOEC from the test. This is likely to be due to the use of non-standard test species and studies of much longer duration than the standard tests as the basis for the modelling.

6 CONCLUSIONS

Predictions of the acute toxicity of the precious metal cyanide compounds, potassium dicyanoargentate and potassium dicyanoaurate, to *Daphnia* have been assessed against testing undertaken on these compounds. This allowed the validity of the speciation calculations and ecotoxicity data for the components to be assessed.

The EC50 for *Daphnia* at pH 8.4 is predicted to occur at a potassium dicyanoargentate concentration of 25.7 $\mu\text{g l}^{-1}$. This prediction is in excellent agreement with the result of the acute toxicity test on *Daphnia*.

The EC50 for *Daphnia* at pH 8.4 is predicted to occur at a silver cyanide concentration of 0.56 $\mu\text{g l}^{-1}$. A comparison of the measured and modelled toxicity of potassium dicyanoargentate suggests that the approach taken to predict the EC50 for silver cyanide is likely to be reliable.

The EC50 for *Daphnia* at pH 8.4 is predicted to occur at a potassium dicyanoaurate concentration of 1.61 mg l^{-1} . A comparison of the measured and modelled toxicity of potassium dicyanoaurate suggests that the approach taken to predict the EC50 for this substance is unlikely to be reliable. There are several possible reasons for the poor prediction of the toxicity of gold cyanide compounds, including the oxidation state of gold following dissolution of this substance, and the ability of VisualMINTEQ to accurately predict the speciation of gold cyanide complexes in aqueous solution.

Testing on tetrachloroauric acid indicates that the toxicity of gold (III) to *Daphnia* is similar to that for fish.

Predictions of the toxicity of potassium dicyanoargentate to STP microorganisms have been assessed against testing results, in order to assess the validity of the approach. The prediction is conservative relative to the measured data by a factor of 45 times. This is due to the use of non-standard ecotoxicity test data in the modelling.

The predicted EC50 and NOEC values for each endpoint are summarised in Tables 5.1 and 5.2, although it should be noted that the predictions for potassium dicyanoaurate are not considered to be reliable.

Further consideration of the ability to accurately predict the speciation of potassium dicyanoaurate solutions would be necessary in order to refine the predictions and with the currently available information it may not be possible to conduct accurate predictions for this substance. Due to the uncertainties with the speciation predictions we would recommend conducting direct testing on fish and algae with potassium dicyanoaurate in order to fill these REACH endpoints, rather than attempting to refine the modelling predictions.

Table 5.1 Predicted acute EC50 values for precious metal cyanide substances.

Substance	Fish (mg l ⁻¹)	Daphnia (mg l ⁻¹)	Algae (mg l ⁻¹)
KAg(CN) ₂	3.3	0.0257	>100
AgCN	3.0	0.00056	80
KAu(CN) ₂ *	<i>0.81</i>	<i>1.61</i>	<i>1.21</i>

*Predictions not considered reliable.

Table 5.2 Predicted NOEC values for toxicity to microorganisms for precious metal cyanide substances.

Substance	Microorganisms (mg l ⁻¹)
KAg(CN) ₂	0.0142
AgCN	0.0094
KAu(CN) ₂ *	<i>Not required</i>

On the basis of the predicted EC50 values for potassium dicyanoargentate and silver cyanide, these substances would be assigned a classification of Acute category 1, Chronic category 1 under the CLP regulation. This is the same as the classification that was submitted for the CLP notification for these substances. The predictions for potassium dicyanoaurate are not considered to be reliable and therefore will not be used for classification purposes. However, based on the measured *Daphnia* result for this substance a classification of Acute category 1, Chronic category 1 would also apply for this substance.

REFERENCES

- Bianchini A, Bowles KC, Brauner CJ, Gorsuch JW, Kramer JR, Wood CM. 2002a. Evaluation of the effect of reactive sulfide on the acute toxicity of silver (I) to *Daphnia magna*. Part 2: toxicity results. *Environmental Toxicology and Chemistry* 21:1294-1300.
- Bianchini A, Gossel M, Gregory S, Wood CM. 2002b. Acute silver toxicity in aquatic animals is a function of sodium uptake rate. *Environmental Science and Technology* 36:1763-1766.
- Bielmyer GK, Grosell M, Paquin PR, Mathews R, Wu KB, Santore RC, Brix KV. 2007. Validation study of the acute biotic ligand model for silver. *Environmental Toxicology and Chemistry* 26:2241-2246.
- Bowles KC, Bianchini A, Brauner CJ, Kramer JR, Wood CM. 2002. Evaluation of the effect of reactive sulfide on the acute toxicity of silver (I) to *Daphnia magna*. Part 1: description of the chemical system. *Environmental Toxicology and Chemistry* 21:1286-1293.
- Brixham Environmental Laboratory. 2012a. Tetrachloroauric acid: Determination of toxicity to the green alga *Pseudokirchneriella subcapitata* (Draft report), Study number: 11-0212/A, Report date: March 2012-04-26
- Brixham Environmental Laboratory. 2012b. Tetrachloroauric acid: Determination of acute toxicity to *Daphnia magna*, Study number: 11-0212/B, Report date: March 2012
- Brixham Environmental Laboratory 2012c Potassium dicyanoargentate: Determination of acute toxicity to *Daphnia magna*, Study number: 11-0213/A, Report date: March 2012
- Brixham Environmental Laboratory 2012d. Potassium dicyanoaurate: Determination of acute toxicity to *Daphnia magna*, Study number: 11-0214/A, Report date: March 2012
- Brixham Environmental Laboratory 2012e. Potassium dicyanoargentate: Activated sludge respiration inhibition test, Study number: 11-0213/B, Report date: March 2012
- Buhl KJ, Hamilton SJ (1991) Relative sensitivity of early life stages of Arctic Grayling, Coho salmon and Rainbow Trout to nine inorganics, *Ecotoxicity and Environmental Safety*, 22, 184-197
- Cairns J, Buikema AL, Heath AG, Parker BC. 1978. Effects of temperature on aquatic organism sensitivity to selected chemicals. Bulletin 106. Virginia Water resources Research Center, Blacksburg, Virginia, USA. (not reviewed)
- Choi O, Hu Z. 2008. Size dependent and reactive oxygen species related nanosilver toxicity to nitrifying bacteria. *Environmental Science and Technology*. 42:4583-4588
- Clements WH, Carlisle DM, Lazorchak JM, Johnson PC. 2000. Heavy metals structure benthic communities in Colorado mountain streams. *Ecological Applications*, 10:626–638.
- DG Environment 2009. State of the art report on mixture toxicity. Final Report. 22 December 2009. Brussels, Belgium.

ECETOC 2007 Cyanides of hydrogen, sodium, potassium, and acetone cyanohydrins (CAS No. 74-90-8, 143-33-9, 151-50-8, and 75-86-5) Volume I. JACC No. 53. Brussels, Belgium.

ECTX. 2012. Transformation / Dissolution test of gold powder at a 1, 10 and 100 mg/L loading in a standard aqueous medium at pH 6. Study No.: X01-061. Report date: 2012-02-3.

Environment Agency 2007 Proposed EQS for Water Framework Directive Annex VIII substances: cyanide (free). Science report SC040038/SR6. Bristol, UK.

Environment Agency 2010 Proposed EQS for Water Framework Directive Annex VIII substances: silver. Science report XXXXXX-EP. Bristol, UK.

Gustafsson 2011 VisualMINTEQ

<http://www2.lwr.kth.se/English/OurSoftware/Vminteq/index.html>

Heugens E, Jager T, Creighton R, Kraak M, Hendriks A, Van Straalen N, Admiraal W. 2003. Temperature-dependent effects of cadmium on *Daphnia magna*: Accumulation versus sensitivity. *Environmental Science and Technology*. 37:2145-2151.

Hirst, H., Jüttner, I. and Ormerod, S.J., 2002. Comparing the responses of diatoms and macroinvertebrates to metals in upland streams of Wales and Cornwall. *Freshwater Biology*, 47:1752–1765.

Lee DR. 1976. Development of an Invertebrate Bioassay to Screen Petroleum Refinery Effluents Discharged into Freshwater. Ph.D. dissertation. Virginia Polytechnic Institute and State University. (not reviewed)

Macissac H, Hebert P, Schwartz S. 1985 Inter- and intraspecific variation in acute thermal tolerance of *Daphnia*. *Physiological Zoology* 58:350-355.

Monsanto. 1981c. Acute toxicity of acetone cyanohydrin to the water flea (*Daphnia magna*). Unpublished report, study BN-81-242. Surprenant DC. EG&G Bionomics, Aquatic Toxicology Laboratory, Wareham, Massachusetts. Monsanto Chemical, St Louis, Missouri, USA [US-EPA/OPTS 87-8216396]. (not reviewed)

Morgan TP and Wood CM. 2004. A relationship between gill silver accumulation and acute silver toxicity in the freshwater rainbow trout; support for the acute silver biotic ligand model. *Environmental Toxicology and Chemistry* 23:1261-1267.

USEPA 1980 Ambient aquatic life water quality criteria for silver. EPA 440/5-80-071. Duluth, Minnesota, USA.

USEPA 1984 Ambient aquatic life water quality criteria for cyanide. EPA 440/5-84-028. Duluth, Minnesota, USA.

US EPA, 1986. Quality Criteria for Water. EPA 440/5-86-001. Office of Water, US Environmental Protection Agency, Washington DC, USA.

USEPA 1987 Ambient aquatic life water quality criteria for silver (Draft 9/24/87). EPA 440/5-87-011. Duluth, Minnesota, USA.

USEPA 2008 TRAP Toxicity Relationship Analysis Program Version 1.20. National Health and Environmental Effects Research Laboratory, Duluth, Minnesota, USA.