# LEAD AND ITS COMPOUNDS

*This EQS dossier was prepared by the Sub-Group on Review of the Priority Substances List (under Working Group E of the Common Implementation Strategy for the Water Framework Directive).*

*The dossier, which is a revision of the original EQS dossier for lead, was reviewed by the Scientific Committee on Health and Environmental Risks (SCHER), whose comments have been addressed as follows.*

*Additional text has been added to section 7 of the dossier to better explain the choice of datasets for deriving the freshwater and marine EQS, and the derivation of summary (geometric mean) toxicity values in these different datasets. The assessment factor used in the SSD approach to deriving a sediment EQS has been specified as 4. The SSD option based on total dissolved lead is retained for pragmatic reasons, since lead appears to be the only metal in the Priority Substances list for which a bioavailability-based sediment EQS exists. Failure to implement that EQS correctly, (i.e. to adequately characterise sediment acid volatile sulfide (AVS) alongside sediment lead) could lead to excessive compliance failure rates. Whilst it is accepted that the back calculation of biota standards to water concentrations is not yet sufficiently robust, the monitoring of lead in biota (particularly with reference to the human health standards in food) can contribute to managing the risks to and via the aquatic environment.*

Introduction

A Voluntary European Union Risk Assessment Report (EU-VRAR) is available for lead (Pb) and two inorganic Pb compounds (Pb oxide, Pb tetraoxide) and Pb stabiliser compounds: a total of thirteen substances in all (LDAI 2008). The risk assessment was thoroughly discussed by EU Member States in the Technical Committee for New and Existing Substances. The EU-VRAR also included an extensive assessment of secondary poisoning and human health.

The EU-VRAR was independently reviewed by the European Commission's Scientific Committee on Health and Environmental Risks (SCHER 2009). Section 2.8.1 of the draft Technical Guidance for Deriving Environmental Quality Standards (EC 2011) recommends that the PNECs derived from the Existing Substances Regulation be adopted as Quality Standards, on the basis that the assessment and the data have undergone thorough peer review. The SCHER specifically commented on the breadth and quality of the aquatic effects database for Pb. However, SCHER concluded that as it had not been possible to account for (bio)availability in the VRAR a reliable PNEC could not be derived. A range of technical issues with the aquatic assessment remained, including the method to be used to account for total Pb data in tests when dissolved data were not available, the use of geometric means, and limited ecotoxicological coverage for certain taxa.

Since the finalisation of the VRAR, the lead industry (International Lead Association Europe – formerly Lead Development Association International, LDAI) has invested significantly in research aimed at addressing both the TCNES and SCHER Opinions. This included ecotoxicity testing for bioavailability correction (laboratory and field studies), water chemistry testing to develop a total-dissolved lead translator, and testing to fill data gaps in the species sensitivity distribution. However, as knowledge and understanding of the fate and behaviour of lead in the aquatic environment developed, in preparation for REACH (EC1907/2006), it has become apparent that the complex chemistry of lead in freshwater may have adversely compromised previous ecotoxicity data. Specifically, processes of chemical precipitation of lead in ecotoxicity tests means that exposures of organisms in some tests previously thought to be valid cannot be calculated. This discovery has profoundly affected the size and quality of the ecotoxicity database for lead.

Nevertheless, the starting point of this factsheet was the EU-VRAR, the SCHER Opinion and a first draft of the Chemical Safety Report for Pb completed to fulfil the REACH requirements provided by the ILA Europe. In addition, the new Technical Guidance has been followed in the EQS derivation process (EC 2011).

The aquatic effects assessment of lead in the EU-VRAR is based on the assumption that adverse effects on aquatic organisms are a consequence of exposure to the available Pb-ion, rather than the parent substances. Effectively this means that the ecotoxicology will be the same for all lead substances that contribute to the formation of the Pb-ion (e.g. Pb metal, Pb oxide, Pb tetraoxide Pb stabiliser compounds, etc). The Environmental Quality Standards derived in this document are relevant for all inorganic Pb substances. Therefore, data from soluble Pb salts are used in the derivation of acute and chronic ecotoxicological values.

# 1 CHEMICAL IDENTITY

| | |
|---|---|
| **Common name** | Lead |
| **Chemical name (IUPAC)** | Lead |
| **Synonym(s)** | - |
| **Chemical class** (when available/relevant) | Metal |
| **CAS number** | 7439-92-1 |
| **EU number** | - |
| **Molecular formula** | Pb |
| **Molecular weight** ($g.mol^{-1}$) | 207.2 |

# 2 EXISTING EVALUATIONS AND REGULATORY INFORMATION

| | |
|---|---|
| **Annex III EQS Dir. (2008/105/EC)** | Not included |
| **Existing Substances Reg. (793/93/EC)** | Lead Metal, Lead Oxide, Lead Tetroxide, Lead Stabiliser, Compounds/ Draft Final VRAR published May 2008 |
| **Pesticides(91/414/EEC)** | Not applicable |
| **Biocides (98/8/EC)** | Not applicable |
| **PBT substances** | Diethyldimethylplumbane, Dioxobis(stearato)trilead, investigated by EU PBT group and both are not considered PBT |
| **Substances of Very High Concern (1907/2006/EC)** | No |
| **POPs (Stockholm convention)** | No |
| **Other relevant chemical regulation (veterinary products, medicament, ...)** | No |
| **Endocrine disrupter** | No |

# 3 PROPOSED QUALITY STANDARDS (QS)

## 3.1 ENVIRONMENTAL QUALITY STANDARD (EQS)

The Generic Environmental Quality Standard for lead is as an $EQS_{available}$. Unless otherwise stated, the other EQS in sections 3.1 and 3.2 are not corrected for (bio)availability.

| EQS | Value | Comments |
|---|---|---|

| | | |
|---|---|---|
| **Proposed AA-EQS$_{available}$ for [freshwater] [µg.L$^{-1}$]**[1]<br>**Proposed AA-EQS for [marine water] [µg.L$^{-1}$]** | 1.2<br>1.3 | **See section 7** |
| **Proposed MAC-EQS for [freshwater] [µg.L$^{-1}$]**<br>**Proposed MAC-EQS for [marine water] [µg.L$^{-1}$]** | 14.25 | **See section 7** |

## 3.2 SPECIFIC QUALITY STANDARD (QS)

| Protection objective | Unit | Value | Comments |
|---|---|---|---|
| Pelagic community (freshwater) | [µg.L$^{-1}$] | Covered by EQS$_{available}$ | **See section 7** |
| Pelagic community (marine water) | [µg.L$^{-1}$] | 1.3 | **See section 7** |
| Benthic community (freshwater) | [mg.kg$^{-1}$ $_{dw}$] | 131 (based on total Pb) or 41 (accounting for bioavailability with AVS/SEM correction) | **See section 7.3** |
| Benthic community (marine) | [mg.kg$^{-1}$ $_{dw}$] | 123 | **See section 7.3** |
| Mammalian predators (secondary poisoning) | [mg.kg$^{-1}$ $_{biota\ ww}$]<br>[µg.L$^{-1}$] | 3.6<br>2.3 | **See section 7.4** |
| Avian predators (secondary poisoning) | [µg.kg$^{-1}$ $_{biota\ ww}$]<br>[µg.L$^{-1}$] | 16.9<br>10.8 | **See section 7.4** |
| Human health via consumption of fishery products | [µg.kg$^{-1}$ $_{biota\ ww}$] | fish muscle meat: 200<br>crustaceans: 500<br>molluscs: 1000<br>cephalopods (excluding viscera): 1000 | **The maximum levels of lead in fishery products intended for human consumption are imposed by Commission Regulation (EC) No 466/2001** |
| Human health via consumption of water | [µg.L$^{-1}$] | 10 | **CD 98/83/EC** |

# 4 MAJOR USES AND ENVIRONMENTAL EMISSIONS

## 4.1 USES AND QUANTITIES

In 2002 in the EU 200,000 tonnes of Pb was produced, but refined metal production was 1,567,000 tonnes and refined metal consumption was 1,733,000 tonnes (LDAI 2008).

[1] The AA-EQS is 1.2 µg Pb.L$^{-1}$ with a dissolved organic carbon (DOC) correction for availability. For Pb, availability is a function of dissolved organic carbon concentration. The incorporation of availability follows the tiered approach that is presented in Section 7.

The EU-VRAR addressed thirteen Pb substances, including Pb metal, Pb oxide, Pb tetraoxide and Pb Stabiliser compounds. Lead metal is mainly used in lead-acid batteries (61%), and in sheet form in the building trade (14%). Lead metal is also used as shot, for alloying and ammunition, in soldering alloys and cable sheathing, and for the production of oxides, pigments, stabilisers and other lead compounds. Lead oxides are mainly used in the EU as PVC stabilising agents and in glass production for televisions and crystal, although other lower tonnage uses include pigments, ceramics and alloys.

## 4.2 ESTIMATED ENVIRONMENTAL EMISSIONS

The total EU emission to surface water from the Pb metal producing sector amounts to 652 kg Pb $year^{-1}$. However, the total emission to surface water from the Pb battery producing sector is approximately 2,400 kg Pb $year^{-1}$. Lead oxide producers discharge 21 kg Pb $year^{-1}$ to surface water and lead stabiliser producers 74 kg Pb $year^{-1}$ (LDAI 2008). Figure 4.1 gives a graphical representation of the key emissions to three environmental compartments. To water the greatest emissions are from households and sewage treatment plants.

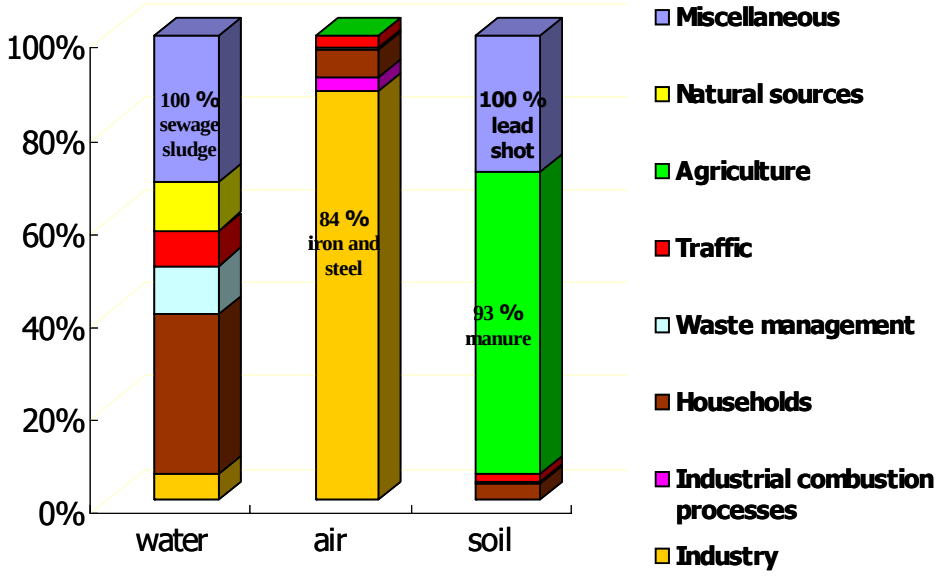


**Figure 4.1. Pb emission sources to water air and soil (LDAI 2008)**

# 5 ENVIRONMENTAL BEHAVIOUR

## 5.1 ENVIRONMENTAL DISTRIBUTION

| Parameter | Value | Master reference |
|---|---|---|
| **Water solubility** ($mg.L^{-1}$) | (Lead metal powder) 185.9 $mg.L^{-1}$<br>[20 °C, at pH = 10.96] | LDAI 2008 |
| **Volatilisation** | | |

| Parameter | Value | Master reference |
|---|---|---|
| **Vapour pressure** (Pa) | 0 mbar at 20ºC<br>1.33 mbar at 1000ºC = 133 Pa | LDAI 2008 |
| **Henry's Law constant** ($Pa.m^3.mol^{-1}$) | Not applicable | |
| **Adsorption** | | |
| **Organic carbon – water partition coefficient ($K_{OC}$)** | Not applicable | |
| **Suspended matter – water partition coefficient ($K_{susp-water}$)** | 295,121 $L.kg^{-1}$ (50th percentile)<br>Range 50,119 - 1,698,244 $L.kg^{-1}$ | LDAI 2008 |
| **$K_{sed}$** | 154,882 $L.kg^{-1}$ (50th percentile)<br>Range 35,481 - 707,946 $L.kg^{-1}$ | LDAI 2008 |
| **Bioaccumulation**<br>**Bioaccumulation Factor (BAF)** | 1554 $L.kg^{-1}$ wwt (mean)<br>440 $L.kg^{-1}$ wwt (50th percentile)<br>Range 7 – 15,400 $L.kg^{-1}$ wwt | LDAI 2008 |
| **Octanol-water partition coefficient (Log Kow)** | Not applicable | LDAI 2008 |
| **BCF (measured)** | 728 :$L.kg^{-1}$ wwt (mean)<br>424 $L.kg^{-1}$ wwt (50th percentile)<br>Range 5 – 8,000 $L.kg^{-1}$ wwt | LDAI 2008 |

## 5.2 ABIOTIC AND BIOTIC DEGRADATIONS

Abiotic and biotic degradation are not relevant parameters for the environmental fate of metals.

# 6 AQUATIC ENVIRONMENTAL CONCENTRATIONS

## 6.1 BACKGROUND

The concentrations of lead in surface waters (both marine waters and freshwater) are variable and depend on both geogenic and anthropogenic sources. Due to these varying exposure conditions, the ambient background concentrations will differ in Europe. As the concentrations measured in the environment are inevitably the sum of both an anthropogenic and a 'natural' component, it is not possible to differentiate easily between the "natural" and the anthropogenic part. Therefore, background concentrations are not measured, but estimated or determined with other methods (EC 2011).

**Pb ambient concentrations in surface waters:**

| Country | Value ($\mu g.L^{-1}$) | Fraction | Mean, median | Reference |
|---|---|---|---|---|
| Finland | 0.07-0.56 | Total | Range | LDAI 2008 |
| Rhine | 0.07 | Dissolved | Mean | |

| | | | | |
|---|---|---|---|---|
| (Germany) | | | | |
| The Netherlands | 0.15 | Dissolved | Mean | |
| Austria | 0.21-0.81 | Dissolved | Range | |
| England | 0.15-3.0 | Total | Range | |
| **General median value** | **0.70**<br>**0.18** | **Total**<br>**Dissolved** | | |
| **England** | 0.43 | Dissolved | 10th Percentile | **Data obtained from the Environment Agency of England and Wales** |
| **Sweden** | 0.41 | Dissolved | Mean | **EIONET** |
| **Data from 23 Member States** | **2.00**<br>**1.00** | **Total**<br>**Dissolved** | **Median**<br>**Median** | James *et al.*, 2009[(1)] |

[(1)] *data originating from EU monitoring data collection*

**Measured or estimated background lead concentrations in European freshwater sediments:**

| Country | Ambient PEC mg.kg$^{-1}$ dry wt | Reference |
|---|---|---|
| Belgium | **17** | LDAI 2008 and references therein |
| Luxembourg | **22** | |
| Northern Sweden | 10 | |
| Swedish west coast | 50 | |
| Swedish reference lakes (50P) | **29** | |
| The Netherlands | 29 | |
| The Netherlands | 21 | |
| The Netherlands | 3 – 28 | |
| The Netherlands | 31 | |
| **The Netherlands - average** | **23** | |
| Norway | **16** | |
| Germany – Elbe | 28 | |
| Germany – Moldau | 32 | |
| Germany – Saale | 24 | |
| Germany – Lake Constance | 23 | |
| **Germany – average** | **26.8** | |
| United Kingdom | 37 – 53 | |
| United Kingdom | 17 – 128 | |
| **United Kingdom - average** | **58.9** | |
| **Median and Range** | **23.5 (16.1 – 58.9)** | |

**Measured or estimated background lead concentrations in the marine environment**

| Country | Concentration | Reference |
|---|---|---|
| ***Seawater*** | | |
| Europe | 0.01 - 0.02 µg.L$^{-1}$ | LDAI 2008 and references therein |
| The Netherlands | 0.02 µg $Pb_{dissolved}$ L$^{-1}$ | |
| North Sea | 0.02 µg $Pb_{dissolved}$ L$^{-1}$ | |
| **Sediment** | | |
| Germany | 25 mg.kg$^{-1}$ dry wt | LDAI 2008 and references therein |
| The Netherlands | 22 - 27 (29)mg.kg$^{-1}$ dry wt<br>37 mg Pb kg$^{-1}$ dry wt | |

# 7 EFFECTS AND QUALITY STANDARDS

Both the VRAR and the SCHER Opinion acknowledge the important influence of water chemistry on the ecotoxicological effects of Pb in the aquatic environment. The most important of these are pH, hardness, and especially dissolved organic carbon (DOC) (Figure 7.1). There are currently no acute or chronic biotic ligand models for Pb, although these are in development. However, there is strong evidence for the mitigating effects of increasing DOC concentrations on Pb toxicity. Statistically significant relationships between DOC and NOEC/$EC_{10}$ values are observed in *Ceriodaphnia dubia* (mortality and reproduction), *Pimephales promelas* (mortality), *Pseudokirchneriella subcapitata* and *Lymnaea stagnalis* (growth) (Figure 7.2). Water hardness and pH have been shown to have a significantly less dramatic effect on Pb availability than DOC (LDAI 2010). Multivariate Spearman Rank Correlation analysis (PRIMER software, version 6) of the influence of DOC, pH and water hardness on Pb NOEC/$EC_{10}$ values in 60 toxicity tests across five species, including *C. dubia*, *Pimephales promelas* and *Pseudokirchneriella subcapitata* consistently identifies DOC as the dominant physicochemical variable influencing the toxicity of lead to freshwater species i.e. DOC alone was able to account for more variability in NOEC/EC10 values than when other parameters were included in the analysis. (Table 7.1).

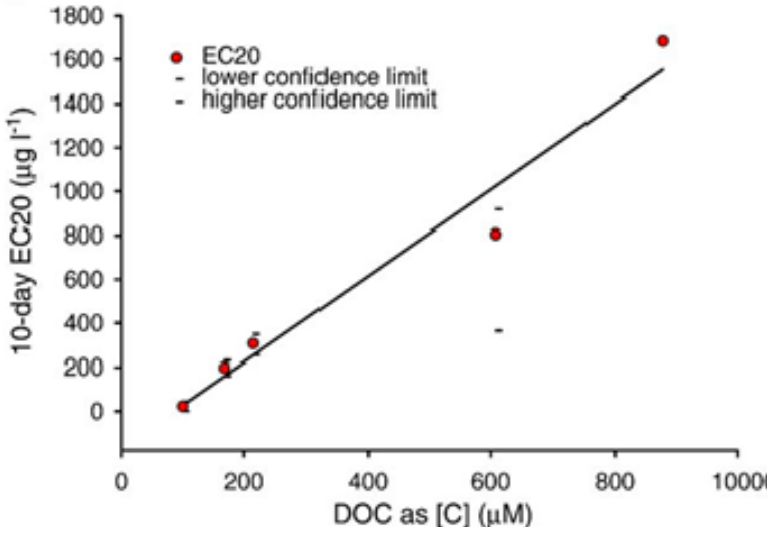


**Figure 7.1** **Effect on Pb toxicity to *P. promelas* under various DOC concentrations. pH and hardness remained constant across the tests (Grosell et al. 2006b)**

**Table 7.1** **Multivariate analysis of influence of water physicochemistry on Pb NOEC/EC10 in various freshwater species.**

| Species | Number of NOEC/EC10 | Correlation Coefficient between NOEC/$EC_{10}$ and water physicochemistry (various permutations of DOC, pH & Hardness) |
|---|---|---|
| *Ceriodaphnia dubia* | 31 | **0.47 (DOC only),** 0.35 (DOC & pH), 0.34 (DOC & hardness), 0.257 (all) |
| *Pimephales promelas* | 10 | **0.91 (DOC only),** 0.64 (DOC & pH), 0.38 (DOC & hardness), 0.32 (all) |
| *Pseudokirchneriella subcapitata* | 7 | **0.80 (DOC only),** 0.79 (DOC & hardness), 0.62 (all) |
| *Philodina rapida* (rotifer) | 5 | **0.77 (DOC only),** 0.65 (DOC & pH), 0.61 (all) |
| *Lemna minor* | 7 | **0.06 (DOC & hardness)**, 0.01 (DOC only) |

Importantly, the data requirements of the VRAR and REACH are different to those for EQS derivation. For the Existing Substances Regulation and REACH the aquatic effects assessment is undertaken with

ecotoxicity data bounded by the 10th to 90th percentile of EU conditions. The WFD is intended to protect all water bodies as far as is practical and will seek to include the more sensitive waters outside the 10th to 90th percentile boundaries. Indeed, the Guidance (EC 2011) for EQS derivation states: "*Use an EQS reference that protects at least 95% of the surface waters instead of 90% in order to follow a precautionary approach*." Therefore, it is inevitable that the PNEC derived under REACH and in the VRAR is different to that derived here. The ecotoxicity data described in Section 7.2 has attempted to include consideration of sensitive waters by not restricting the tests used to the 10-90th percentile physicochemical boundaries.

The strong relationship between DOC and chronic Pb toxicity to aquatic organisms provides an opportunity to explore the derivation and implementation of a Pb EQS with account taken of "availability" as outlined in the Technical Guidance (EC 2011).

The approach taken assumes that DOC-bound Pb is not bioavailable. The concentrations of the free metal ion and total soluble species were calculated for Pb ecotoxicity tests using VisualMINTEQ and WHAM. Both the free metal ion activity and the total concentration of truly soluble species were considered as it may be that simple inorganic forms of Pb, other than the free metal ion, may be contributing to the observed toxic effect. Considering the total soluble forms did reduce the inter-test variability relative to the free metal ion approach, but not by much. In summary, there is considerable evidence that DOC reduces the chemical availability of Pb but insufficient evidence to propose a biotic ligand based model that includes other physicochemical variables. Therefore, an approach which simply considers the effect of DOC on the response of organisms to lead toxicity in laboratory tests has been evaluated and the results are shown here.

There are six species in the freshwater ecotoxicity database for which tests have been performed at multiple DOC concentrations, and therefore the effect of DOC concentration on lead toxicity can be considered. For two of these species (*C. dubia* and *Lymnaea stagnalis*) the data were split into two sets covering different endpoints, resulting in eight analyses of the effect of DOC on lead toxicity. A summary of linear (least-squares) regression analyses on these datasets is shown in Table 7.2, and Figure 7.2. In the majority of cases a linear relationship was observed between the DOC concentration in the test and the observed EC10 (or NOEC) which was significant at the 95% confidence level. Many of the species assessed showed a very strong effect of DOC in reducing lead toxicity, with slopes as high as 142 $\mu g.L^{-1}$ Pb EC10 per $mg.L^{-1}$ DOC for *P. promelas*. However, such steep slopes were not observed in all species e.g. *Philodina rapida* and *P. subcapitata* showed a less protective effect of DOC .

**Table 7.2 Regression analysis of DOC concentration in test media versus Pb NOEC/EC10 for various freshwater species**

| Species | Endpoint | n | Slope | SE | p | $r^2$ (adj) | DOC range | DOC Factor |
|---|---|---|---|---|---|---|---|---|
| *C. dubia* | Mortality | 19 | 53.9 | 73.19 | <0.0001 | 0.66 | 0.5 – 7.2 | 14.4 |
| *C. dubia* | Reproduction | 31 | 15.6 | 48.93 | <0.0001 | 0.56 | 0.5 – 17.3 | 34.6 |
| *P. promelas* | Mortality | 10 | 142.2 | 196.76 | 0.0001 | 0.84 | 1.2 – 10.5 | 8.75 |
| *L. minor* | Growth rate (N° of fronds) | 7 | 40.4 | 299.26 | 0.2148 | 0.14 | 0.5 – 12.5 | 25 |
| *P. subcapitata* | Growth rate | 7 | 4.6 | 16.25 | 0.0087 | 0.73 | 1.8 – 17.4 | 9.67 |
| *P. rapida* | Population growth | 5 | 1.2 | 5.70 | 0.0670 | 0.63 | 0.9 – 16.9 | 18.8 |
| *L. stagnalis (Grosell, 2010b)* | Growth (weight) | 4 | 8.7 | 6.44 | 0.0084 | 0.97 | 6.3 – 15.8 | 2.5 |
| *L. stagnalis (Parametrix, 2007)* | Growth (wet weight) | 2 | 2.5 | - | - | - | 0.5 – 7.1 | 14.2 |

The relationship with the lowest slope between DOC concentration and EC10 was observed for the rotifer *P. rapida*, derived from five tests covering an 18-fold difference in DOC concentrations. The relationship between DOC and NOEC/EC10 was not quite statistically significant at the 95% level ($p = 0.0670$), which was considered to be due to the variability of other physicochemical parameters, which confounded the DOC relationship (e.g. hardness and pH, which ranged from 5-133 $mg.L^{-1}$ and 7.2-8.4 across the tests, respectively). The slope derived for the relationship between EC10 and DOC concentration was 1.2 ($\mu g.L^{-1}$ Pb EC10 per $mg.L^{-1}$ DOC). The value of 1.2 is used in Equation 1 below.

No significant relationship was observed for the effect of DOC on the growth rate of *L. minor*, although this was also considered to be due to the variability of other physicochemical parameters which confounded the DOC relationship (e.g. hardness and pH) rather than DOC concentration not affecting toxicity. In addition, *L. minor* is significantly (approximately an order of magnitude) less sensitive to Pb exposure than other species, under the majority of the water conditions tested. Taking a conservative approach towards deriving a slope still resulted in a steeper slope function than was observed for *P. rapida*.

Therefore, *P. rapida* is considered to be the species whose sensitivity to lead is least affected by the DOC concentration. The proposal for a precautionary DOC correction on the reference PNEC is based on the response of this species, despite the fact that the regression was not statistically significant at the 5% level (however it was significant at 6.7%). This approach assumes that there will not be any species in natural freshwater ecosystems for which the relationship between DOC concentration and EC10 would have a lower slope than that derived for *P. rapida*. This assumption is untested, but a greater slope (up to 118 times greater) has been observed for five other species for which ecotoxicity tests under different DOC regimes are available. Using the lowest slope observed in the ecotoxicity dataset is the most precautionary form of linear DOC correction that can be applied. Use of a steeper slope could potentially result in some species not being protected at higher DOC concentrations.

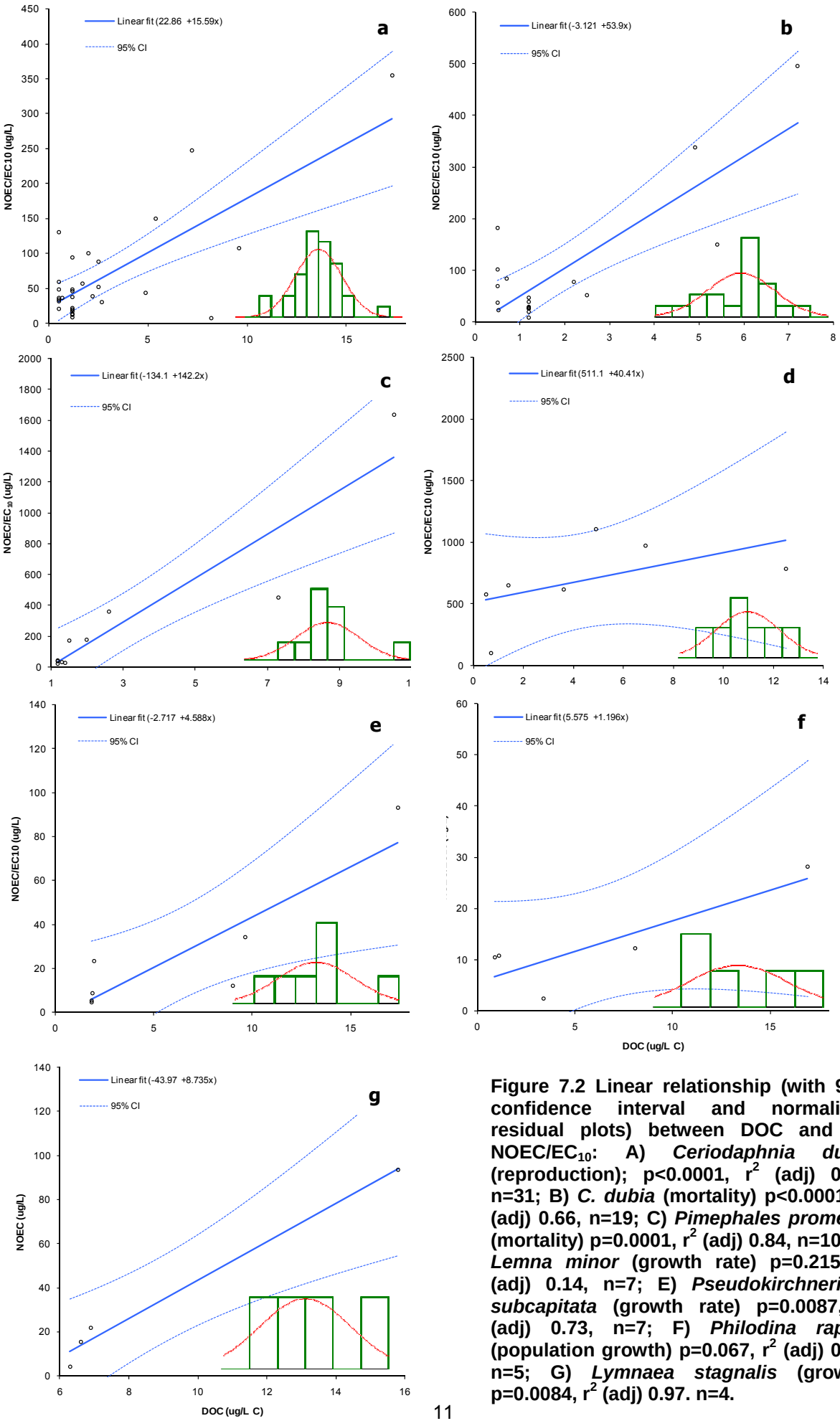


**Figure 7.2 Linear relationship (with 95% confidence interval and normalised residual plots) between DOC and Pb NOEC/$EC_{10}$: A) *Ceriodaphnia dubia* (reproduction); p<0.0001, $r^2$ (adj) 0.56, n=31; B) *C. dubia* (mortality) p<0.0001, $r^2$ (adj) 0.66, n=19; C) *Pimephales promelas* (mortality) p=0.0001, $r^2$ (adj) 0.84, n=10; D) *Lemna minor* (growth rate) p=0.215, $r^2$ (adj) 0.14, n=7; E) *Pseudokirchneriella subcapitata* (growth rate) p=0.0087, $r^2$ (adj) 0.73, n=7; F) *Philodina rapida* (population growth) p=0.067, $r^2$ (adj) 0.63, n=5; G) *Lymnaea stagnalis* (growth) p=0.0084, $r^2$ (adj) 0.97. n=4.**

A site-specific PNEC or EQS can therefore be calculated according to Equation 7.1. An alternative approach would be to use the species with the shallowest response to DOC which was statistically significant at the 5% level. The alga *P. subcapitata* has a statistically significant ($p<0.05$) slope of 4.6, approximately four times steeper than *P. rapida.* Using this approach would result in a less stringent standard that may not be protective of species that show a lesser protective effect of DOC i.e. *P. rapida*. We do not recommend this approach.

$$\mathbf{PNEC_{site} = PNEC_{reference} + (1.2 \times (DOC - DOC_{reference}))} \qquad \textbf{Eq. 7.1}$$

*Where:*

$PNEC_{site}$ = is the Predicted No Effect Concentration at the site under consideration

$PNEC_{reference}$ (or Generic or Reference EQS) = EQS for a reference condition to ensure all water bodies are protected.

DOC = Dissolved Organic Carbon at the site under consideration

$DOC_{reference}$ = average Dissolved Organic Carbon (DOC) concentration in the ecotoxicity tests that the $PNEC_{reference}$ is based upon, 1.0 $mg.L^{-1}$.

As outlined in the EQS Guidance (Sections 3.2.5.1 and 3.2.5.2) this equation can then be fed into the following calculation in order to derive a BioF:

*$BioF = QS_{reference}$ (1.0 $mg \cdot l^{-1}$ DOC)/$QS_{site\text{-}specific}$ (normalised to the site-specific $mg \cdot l^{-1}$ DOC.*

*Determine the available dissolved metal concentration at the site, calculated as dissolved metal concentration $\times$ BioF.*

This calculation corrects the measured Pb exposure in the sample into an “available” Pb exposure. This approach partly accounts for the physicochemical aspects of bioavailability, which can be considered to be a combination of the physicochemical factors governing metal behaviour and the biological receptor - its specific pathophysiology. The advantage of using the BioF in the way described above is that there is only a need for one Pb EQS across all freshwaters.

The predicted $PNEC_{site}$ derived from a DOC correction in equation 1 was compared to the modelled free ion activity approach reported in the lead REACH CSR. The dissolved Pb concentration required to maintain a constant free Pb ion activity over a range of DOC concentrations was calculated using VisualMINTEQ with initial physicochemical conditions fixed at pH 7.6, and hardness 53.6 mg $l^{-1}$. Other physicochemical variables (Mg, Na, K, Cl, $SO_4$, and alkalinity) were estimated from a correlation with Ca, as used in the CSR.

Both approaches produce almost identical predictions of $EQS_{Pb\ dissolved}$ (Figure 7.3). The similarity of the outputs from the CSR speciation modelling provides supporting mechanistic evidence to the proposed approach based on observed NOECs/EC10s. Similar results are also obtained when WHAM is used to perform the speciation calculations.

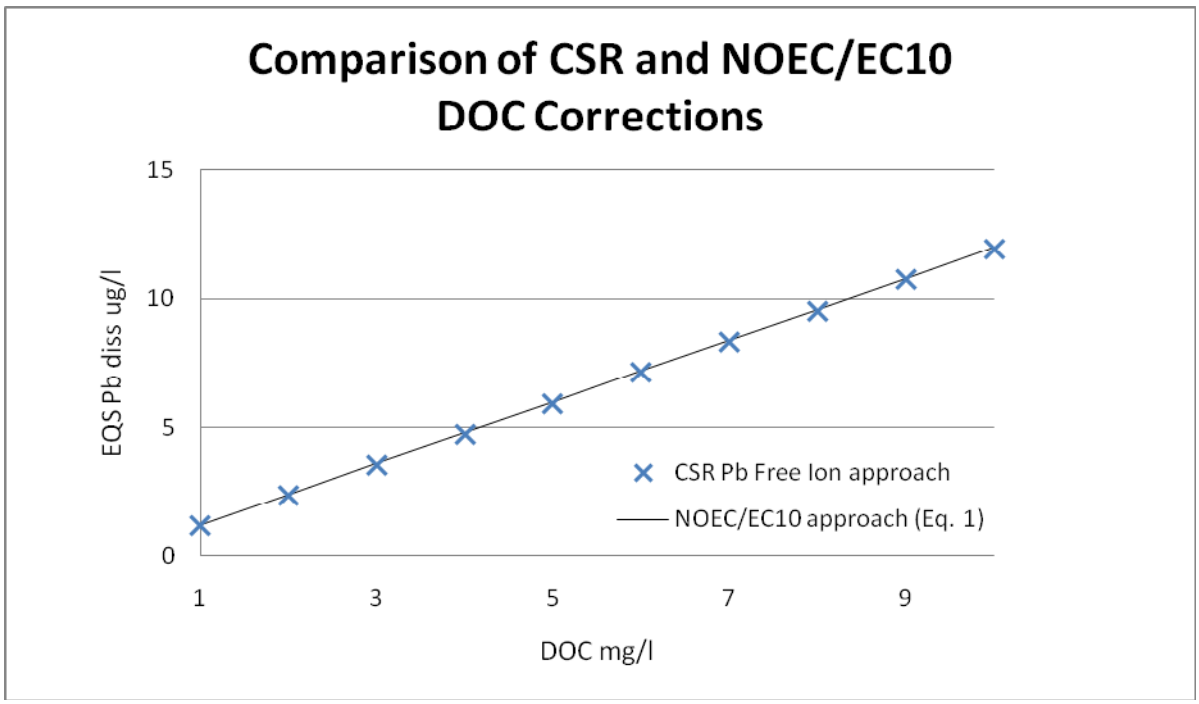


**Figure 7.3. Calculated $EQS_{Pb\ Dissolved}$ for increasing DOC concentrations using the outputs from Equation 1 and the speciation modelling approach in the CSR**

The EC EQS Technical Guidance has provided great clarity on the implementation of bioavailability-based approaches for metals (EC 2011). The guidance advocates the use of a tiered approach (e.g. Figure 7.4). The first tier involves the use of a generic or reference EQS (without bioavailability) with subsequent tiers incorporating some element of bioavailability correction. Applying a DOC correction to the generic Pb EQS results in an $EQS_{available,}$ which is then compared to the annual average of the dissolved Pb monitoring databeyond tier one. The generic Pb $EQS_{available}$ should be protective for *all* water bodies that may be monitored (Section 7.2).

The proposed approach uses an empirical methodology which is based on the effect of DOC on the NOEC/EC10 values of the species for which lead toxicity appears to be least affected by increases in DOC concentration. Importantly, the generic Pb EQS is *inseparable* from the tiered availability-based approach. This is effectively the same as defining an EQS on the basis of a specific form of a chemical, such as for other WFD EQS, e.g. un-ionised ammonia, reactive aluminium, or free chlorine. The correction being advocated here is very similar to that currently being applied with the existing EQS Cd in relation to hardness, and to that used for Cu with DOC by the Rhine Commission and in the STOWA work (Zwolsman and De Schamphelaere 2007).

In addition, the suitability and robustness of the proposed availability correction for lead has been appraised in detail against the requirements of the OECD guidance on the validation of QSAR (or related ) models for regulatory purposes (OECD 2007). The five principles for consideration (i.e. Defined endpoint, defined algorithm, defined domain of applicability, internal performance and predictivity) are considered in detail in Annex 3. All requirements outlined for each of the principles are considered to have been met by the proposed availability correction for lead.

## 7.1 METHODOLOGY FOR THE IMPLEMENTATION OF AN AVAILABILITY CORRECTION FOR LEAD

The proposed AA EQS for Pb is an $EQS_{available}$ incorporating the DOC correction as discussed above. In order to facilitate the implementation of this approach, and in accordance with the Guidance, a simple Microsoft Excel-based™ Screening Tool for Pb has been developed that can perform the DOC correction calculations. The DOC correction for Pb is effectively a simple "availability" correction. The Pb Screening Tool predicts site-specific availability of Pb from DOC and the dissolved Pb concentration. Importantly, the basis for the calculation is very simple and can be incorporated into laboratory data management systems, so the tool can be readily automated.

The Screening Tool has been developed for use as an early tier in a tiered risk-based compliance framework (Figure 7.4). Using the generic $EQS_{available}$ as derived in Section 7.2 the Screening Tool calculates a bioavailability factor (BioF). This BioF is then applied to the measured monitoring data to give the available concentration of Pb at the specific site under consideration. This "available" Pb concentration is then compared to the generic Pb $EQS_{available}$. An example of a tiered compliance framework to account for Pb availability is shown in Figure 7.4. This follows a standard risk assessment paradigm in which early tiers are conservative, but allow high relative sample throughput (Environment Agency 2009). The lowest tier of this assessment is precautionary and uses the conservative generic $EQS_{available}$. Subsequent tiers also require information on DOC concentration and measured dissolved Pb at monitoring/compliance sites. If there is to be adoption of (bio)availability-based compliance assessment frameworks for metals it is important to ensure that either the same or fewer resources are required than for existing approaches to compliance assessment (UBA 2008). The individual tiers in the framework are described below as detailed by the Environment Agency (2010). The processes undergone beyond Tier 3 are not discussed here and would be the concern of individual Member States.

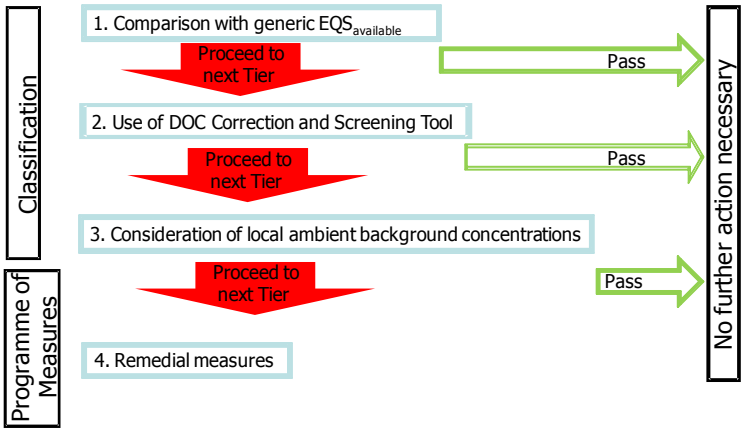


**Figure 7.4. Flow diagram of the stages in a tiered compliance assessment. The red arrows indicate sites that continue through the tiered process, green arrows are sites that need no longer be considered as having a potential lead risk (Environment Agency 2009; EC 2011).**

**Tier 1**. The first tier in the scheme is a direct comparison between the annual average concentration from monitoring data with the generic "available" Pb EQS. The EQS is expressed as an "available" concentration, but is initially compared to the dissolved Pb measurement. This results in a relatively precautionary assessment in which false negatives (Type II errors) are minimised. A Generic $EQS_{available}$ of 1.2 $\mu g.L^{-1}$ dissolved Pb is proposed as being sufficiently protective of relatively high bioavailability conditions (Section 7.2). Sites, or samples, giving a risk characterisation ratio (RCR) of equal to or greater than 1 at this tier proceed to the second tier of the assessment. At Tier 2 data on DOC concentrations are required as an input to the Screening Tool..

**Tier 2**. This tier uses an Excel™-based Screening Tool to perform the DOC correction. Samples with an RCR equal to or greater than 1 proceed to Tier 3 and the consideration of local ambient background concentrations. Tier 2 requires information on the site DOC concentration of the waterbody/sample point, although in some cases default values for DOC may be available (Environment Agency 2009).

**Tier 3.** This tier considers the use of specific localised ambient natural background concentrations (ABCs). The use of waterbody or hydrometric area-specific Pb ABCs for which (bio)availability corrections exist (such as copper, nickel, lead or zinc) is expected to be limited because of the exclusion of locations requiring attention during earlier tiers of the assessment. The uncertainty associated with the derivation and use of ABCs is significantly greater than uncertainty from the use of the DOC correction, and therefore ABCs must only be considered after the use of the availability models. Indeed, the use of backgrounds in compliance assessment using the "added risk approach" is a pragmatic and not scientifically driven decision (EQS Technical Guidance (EC 2011)). In many cases the application of a background correction to sites which have reached this tier of the assessment is unlikely to result in a change in the conclusion of the assessment. This is especially true if (bio)availability has been taken into account, due to the relatively low level of background concentrations in much of Europe (Environment Agency 2008).

## 7.2 ACUTE AND CHRONIC AQUATIC ECOTOXICITY

### Acute toxicity

For the acute Pb data there is no opportunity to be able to make a correction for (bio)availability. Unlike the freshwater chronic dataset, there is not the depth of understanding in terms of the influence of abiotic factors on acute Pb toxicity. As such a standard approach for the derivation of a short-term QS (or Maximum Acceptable Concentration: MAC) has been adopted. Freshwater and marine datasets were combined for the derivation of the MAC as there was no statistically significant difference between the means of $\log_{10}$ transformed datasets ($p>0.05$). Where several LC/EC50 values were available for a species a geometric mean of these values has been used.

**Table 7.3 Summary of the $LC/EC_{50}$ values (total risk approach) in µg $Pb.L^{-1}$ for freshwater and saltwater organisms (n=31):**

| Taxonomic group | Species | Habitat | LC/EC50 value (µg.$L^{-1}$) |
|---|---|---|---|
| Alga | *Skeletonema costatum* | SW | 72.7 |
| | *Chlorella stignatophora* | SW | 100 |
| | *Minutocellus polymorphus* | SW | 1000 |
| | *Dunaliella tertiolecta* | SW | 1231.8 |
| | *Pseudokirchneriella subcapitata* | FW | 80.3 |
| Annelid | *Tubifex tubifex* | FW | 200.5 |
| Crustacean | *Ceriodaphnia dubia* | FW | 314.19 |
| | *Alona rectangula* | FW | 5260 |
| | *Daphnia carinata* | FW | 444 |
| | *Diaphanosoma birgei* | FW | 2360 |
| | *Moina micrura* | FW | 2410 |
| | *Cancer magister* | SW | 600 |
| Echinoderm | *Dendraster excentricus* | SW | 569.9 |
| | *Strongylocentrotus droebachiensis* | SW | 19000 |
| | *Strongylocentrotus franciscanus* | SW | 1300 |
| | *Strongylocentrotus purparatus* | SW | 957.5 |
| Fish | *Clarias lazara* | FW | 1720 |

| Taxonomic group | Species | Habitat | LC/EC50 value ($\mu g.L^{-1}$) |
|---|---|---|---|
| | *Micropterus dolomieui* | FW | 2800 |
| | *Oreochromis nilotius* | FW | 2150 |
| | *Pimephales promelas* | FW | 465.6 |
| | *Onchorhynchus mykiss* | FW | 1000 |
| | *Scorpaenichtys marmoratus* | SW | 1500 |
| Insect | *Benacus* sp. | FW | 1890 |
| | *Chironomus tentans* | FW | 2680 |
| Mollusc | *Lampsilis siliquoidea* | FW | 142 |
| | *Lampsilis rafinesqueana* | FW | 298 |
| | *Mytilus edulis* | SW | 25 |
| | *Mercenaria mercenaria* | SW | 1000 |
| | *Crassostrea gigas* | SW | 185.6 |
| | *Mytilus galloprovincialis* | SW | 263.8 |
| Protozoan | *Navicula incerta* | SW | 100 |

FW=freshwater; SW=seawater

Given the number and taxonomic spread of the ecotoxicity data, a statistical approach was used to derive a 5$^{th}$ percentile Hazardous Concentration ($HC_5$).

Data were analysed using RIVM ETX 2.0 (http://www.rivm.nl/rvs/risbeoor/Modellen/ETX.jsp) software for deriving SSDs. Figure 7.5 shows the graphical output from the ETX lognormal model fitted to the data. The $HC_5$ is 57.1 $\mu g.l^{-1}$ (confidence interval (90%) = 25.8 – 103.0 $\mu g.l^{-1}$). Anderson Darling, Cramer von Mises and Kolmogorov-Smirnov tests for goodness of fit (GoF) were all met at the 0.05 significance level.

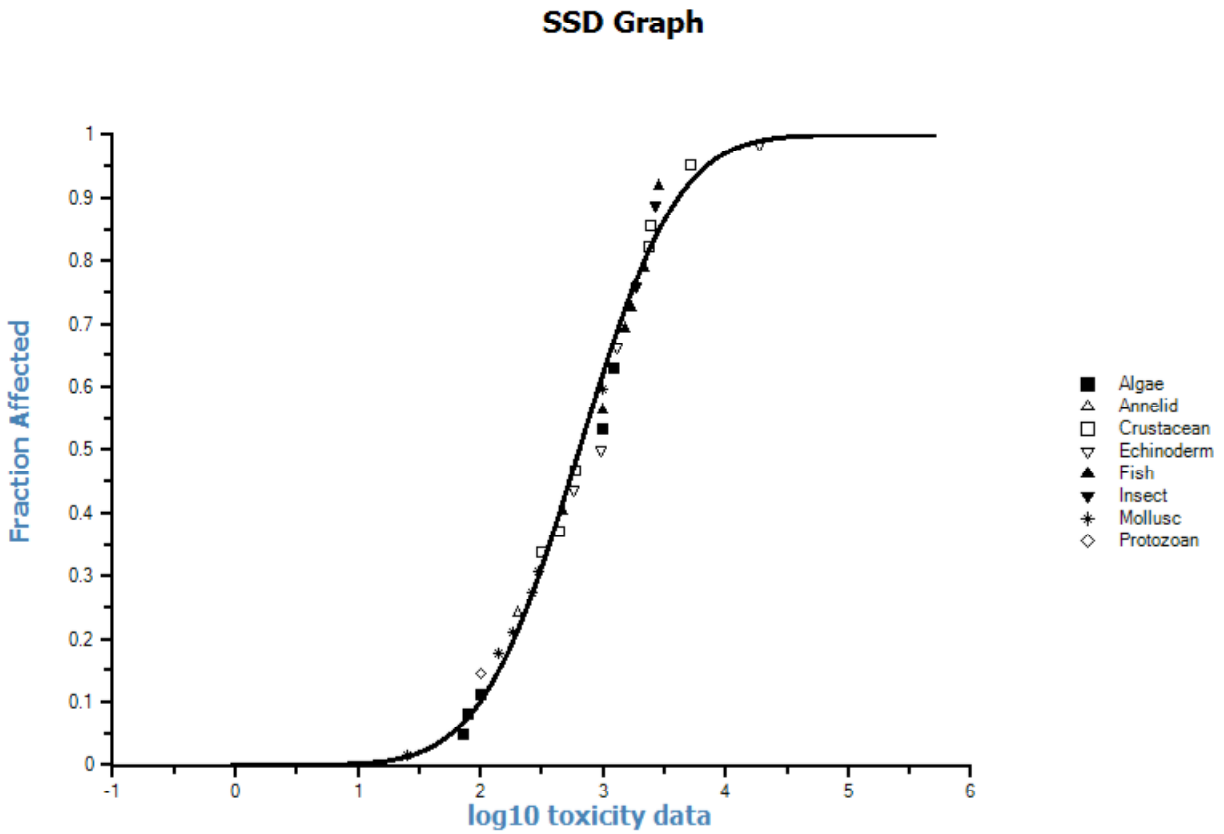


**Figure 7.5** **Lognormal Species Sensitivity Distribution for lead based on n=31 acute aquatic data points generated using ETX.**

The following conclusions can be drawn:

1. The default assessment factor in the EQS technical guidance to be applied to the HC5 derived from acute data is 10. However, the number of data points for Pb across a range of species (31 separate species values), and higher taxonomic groups (8) means that a large assessment factor need not be applied to this $HC_5$. An AF of 4 on the HC5 from the combined dataset, to produce a MAC of 14.25 $\mu g.l^{-1}$, is appropriate for the following reasons:
    a. The data-set contains information on 28 species representing 8 higher taxonomic groups.
    b. The proposed MAC is below the lower confidence limit (25.8 $\mu g.l^{-1}$) from the ETX analysis.
    c. The lowest value in the combined acute dataset is for the saltwater mollusc *Mytilus edulis* (25 $\mu g.l^{-1}$), which would not be exceeded by the proposed MAC.

## Chronic toxicity

All available chronic toxicity data for lead (including industry funded studies) have been collated and reliability assessed as part the recent VRAR for lead and, more recently, for REACH registration. There is a reasonably large set of reliable (K1 and K2) data on the chronic aquatic toxicity of soluble lead in freshwater, including the major taxonomic groups, i.e. algae, crustaceans and fish. Additional data for a greater number

of species (but not taxa) are available in studies reporting effects based on total Pb exposure. However, total Pb data are not considered directly applicable to the derivation of a bioavailability-based EQS without a reliable relationship for the conversion between dissolved and total Pb concentrations in ecotoxicity tests (as discussed in the TGD-EQS (EC 2011)). As such a relationship is not currently available (despite the availability of a limiting function for Pb solubility), ecotoxicity data expressed as total Pb are not considered for use in EQS derivation here. The ecotoxicity dataset based solely on studies reporting dissolved Pb remains sufficiently large (in terms of both species and taxonomic spread) for robust EQS derivation. In addition, as it is intended to account for the bioavailability of lead during PNEC derivation, at least in the freshwater environment, chronic toxicity data for marine species are not included in this dataset and a marine EQS has been derived separately. This is consistent with section 3.5.2 of the TGD-EQS which states that "corrections for freshwater cannot currently be directly translated to saltwater conditions; therefore, pooling of freshwater and saltwater data should be avoided when availability corrections have been applied".

The relationship between water physicochemistry and Pb bioavailability has yet to be precisely defined. However, as the bioavailability and corresponding toxicity of lead is known to be influenced by test media physicochemistry (including pH, hardness and DOC) where there were several NOEC/$EC_{10}$ values available for a species, the $EC_{10}$/NOECs used for EQS derivation were restricted, where possible, to those from tests that were conducted under physicochemical conditions consistent with "reasonable worst case" maximum Pb bioavailability (low DOC, low hardness, low to moderate pH). Tests with "similar" physicochemistry consistent with "reasonable worst case" maximum Pb bioavailability were identified from a wider toxicological dataset using principal components analysis (Annex 2). The geometric mean of NOEC/EC10 values from "similar" tests was taken as the respective species mean NOEC/$EC_{10}$ value. By using such an approach tests that were conducted under low DOC conditions, but which may have pH or hardness characteristics that would have limited pH bioavailability are excluded from EQS derivation. The EQS derived from a "reasonable worst case" dataset is considered to be a $EQS_{refrence.}$

The physicochemistry across the resulting "reasonable worst case" toxicological dataset corresponds to a mean DOC concentration of ~1.0 mg $C.L^{-1}$ (maximum 1.9 mg $C.L^{-1}$), a mean pH of 7.56 (maximum 8.4) and mean hardness of 53.6 $mg.L^{-1}$ (maximum 138.0 $mg.L^{-1}$). In terms of DOC, 1.0 mg $C.L^{-1}$ corresponds to approximately the $4^{th}$ percentile of conditions encountered in the UK. The mean hardness is close to the $40^{th}$ percentile of UK conditions and the mean pH is close to the $50^{th}$ percentile of UK conditions.

In terms of toxicity, the taxa most sensitive to Pb are molluscs (represented by *L. stagnalis*), followed by algae (*P. subcapitata*) and *Hyalella azteca* (a species of amphipod crustacean). Fish (both salmonid and cyprinid) would appear to be relatively insensitive to Pb in comparison to invertebrates, especially molluscs. Plants and chironomid larvae are the most insensitive taxa in the species sensitivity distribution.

**Table 7.4 Summary of the "species mean" NOEC or $EC_{10}$ values (total risk approach) in µg dissolved $Pb.L^{-1}$ (with most sensitive endpoint) for freshwater organisms (n=10).**

| Taxonomic group | Species | Most sensitive endpoint | NOEC/$EC_{10}$ (µg dissolved $Pb.L^{-1}$) | Test physicochemistry[4] | | |
|---|---|---|---|---|---|---|
| | | | | DOC ($mg.L^{-1}$ C) | pH | Hardness ($mg.L^{-1}$) |
| **Algae** | *Pseudokirchneriella subcapitata*[1] | Growth rate | 8.42 | 1.9 | 7.2 | 24.2 |
| **Higher plants** | *Lemna minor*[1] | Growth rate | 104.0 | 0.7 | 7.9 | 29.0 |
| **Rotifer** | *Brachionus calyciflorus*‡ | Population growth | 89.5 | 0.5[2] | 7.8 | 128.0 |
| | *Philodina rapida*[1] | Population growth | 10.66 | 1.0 | 7.7 | 107.5 |
| **Molluscs** | *Lymnaea stagnalis*† | Growth | 1.7 | 0.5 | 7.3 | 83.0 |
| **Crustaceans** | *Ceriodaphnia dubia*[1] | Reproduction | 36.78 | 1.2 | 7.2 | 32.1 |
| | *Hyalella azteca*Ω | Growth | 8.2 | 1.1 | 8.4 | 138.0 |
| **Insects** | *Chironomus tentans*‡ | Emergence | 109.0 | 1.2 | 7.9 | 46 |
| **Cyprinid fish** | *Pimephales promelas*[1] | Mortality | 29.29 | 1.3 | 7.1 | 26.8 |
| **Salmonid fish** | *Salvelinus fontinalis*Ψ | Weight | 39.4 | 1.0[3] | 7.2 | 44.3 |
| | | | | | | |
| **Mean** | | | | **0.96** | **7.59** | **53.55** |

1: Species NOEC/EC10 based on a geometric mean of the results of multiple tests with comparable methodology and "similar" physicochemistry. See Annex 2 for further detail and reference information.
2: DOC estimated as 0.5 $mg.L^{-1}$ from typical values for reconstituted media.
3: DOC of Lake Superior water was assumed to be 1 $mg.L^{-1}$ C (following Erickson et al., 1996).
4: Variability of DOC and hardness across tests is summarised as a geometric mean. Variability of pH across tests is summarised as an arithmetic mean
‡: Grosell et al., 2006a
Ω: Besser et al., 2005
†: Parametrix, 2007
Ψ: Holcombe et al., 1976

As there are sufficient data available (criteria for 10 NOEC/$EC_{10}$ values across a minimum of eight taxonomic groups are met), a statistical approach (SSD) was used to derive a 5th percentile Hazardous Concentration ($HC_5$).

Data were analysed using RIVM ETX 2.0 (http://www.rivm.nl/rvs/risbeoor/Modellen/ETX.jsp) SSDs. Figure 7.6 shows the graphical output from the ETX lognormal model fitted to the data. The $HC_{5\text{-}50}$ is 2.35 $\mu g.L^{-1}$ (confidence interval (90%) = 0.45 – 5.94 $\mu g.L^{-1}$). All statistical tests for goodness-of-fit (Anderson-Darling, Kolmogorov-Smirnov and Cramer von Mises) were met.

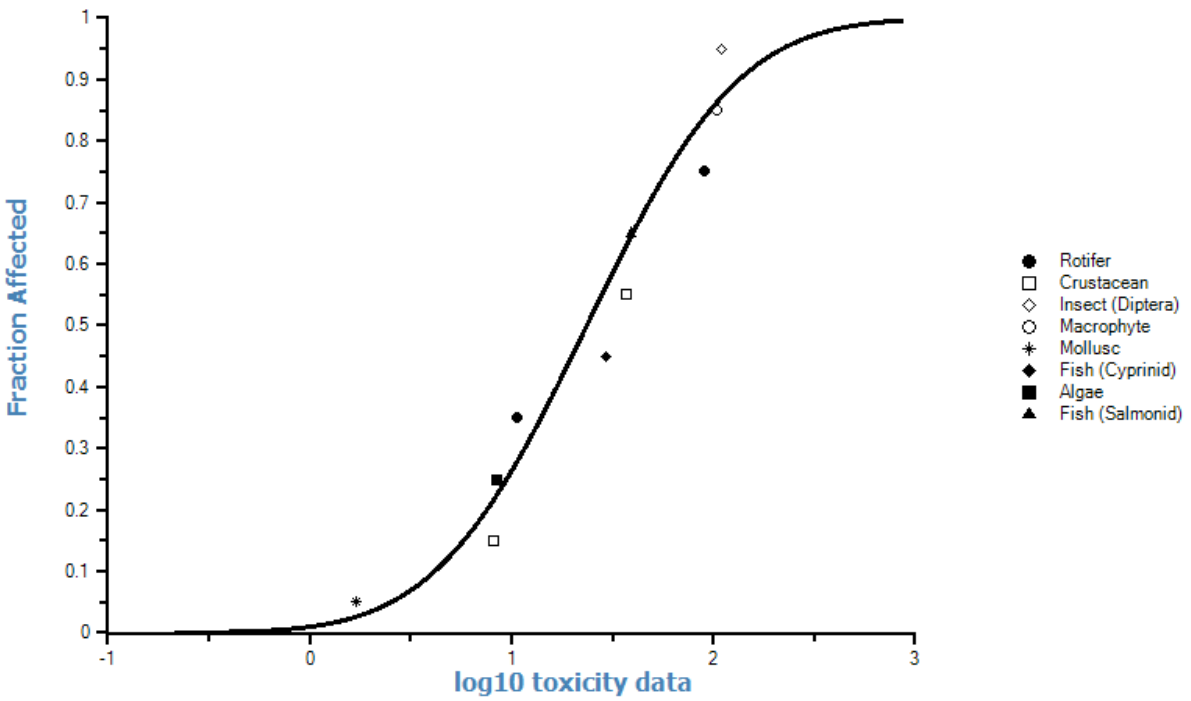


**Figure 7.6 SSD for long-term toxicity of dissolved Pb to freshwater organisms.**

An assessment factor between 1 and 5 should be applied to the 50% confidence value of the 5$^{th}$ percentile value (i.e. EQS = HC5/AF). The AF selected is based on the confidence in the estimation of the HC5 and the likelihood of residual uncertainty that might give rise to risks that are not adequately accounted for in the extrapolation and estimation of the HC5.

Application of an assessment factor of 2 results in an $PNEC_{reference}$ for dissolved lead of 1.18 µg.L$^{-1}$. This is considered to be appropriate for the following reasons:

a. The ecotoxicity dataset meets the minimum acceptability criteria (London Workshop Criteria) in the WFD and REACH technical guidance for both the number of NOEC/EC10 values and taxonomic spread. Chronic NOEC/EC10 values are available for algae, higher aquatic plants, two species of rotifer, molluscs, two species of crustaceans, insects and two families of fish (cyprinids and salmonids).
b. The dataset used for HC5-50 derivation is preselected from the wider toxicological dataset to reflect "reasonable worst case" bioavailability conditions for Pb in the aquatic environment. The mean DOC of the "reasonable worst case" ecotoxicological dataset (1.0 mg C.L$^{-1}$) is consistent with the protection of >95% of waterbodies in the UK and the wider EU. The selection of a larger assessment factor to account for conditions of high bioavailability is not necessary.
c. The lowest value in the overall freshwater chronic dataset, by a significant margin, is for the mollusc *Lymnaea stagnalis* (1.7 µg.L$^{-1}$), which would not be exceeded by the proposed $PNEC_{reference}$. The second most sensitive datum is for the amphipod Crustacean *Hyalella azteca* (8.2 µg.L$^{-1}$). The lowest reported $NOEC/EC_{10}$ for a species of fish is 29.29 µg.L$^{-1}$ (*Pimephales promelas*).
d. Analysis of field data does not support the application of a more stringent AF (see below).
e. The availability correction based on DOC is applied in a precautionary manner (see Figure 7.7), which results in a threshold which is protective of 98% of the available ecotoxicity data (for further details refer to annex 3).

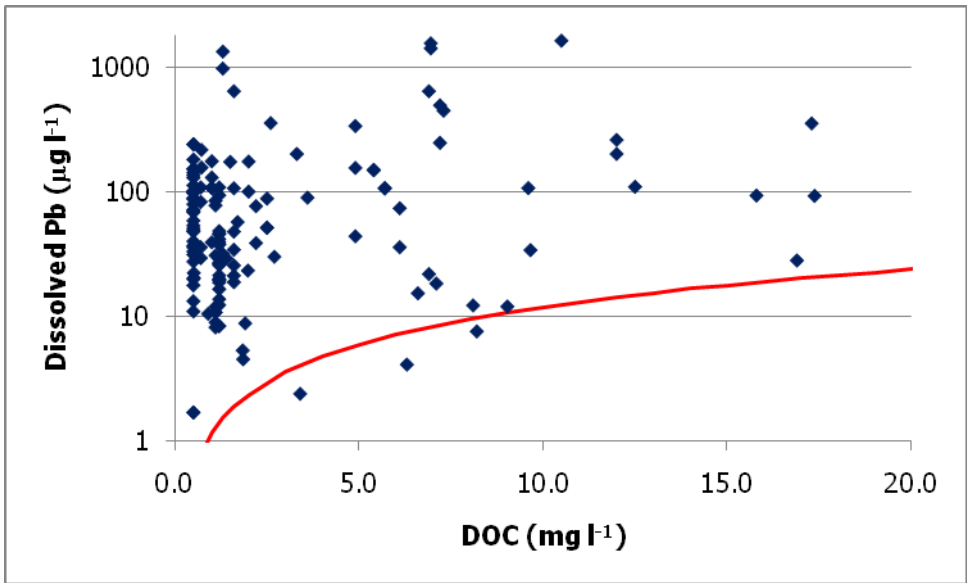


**Figure 7.7 Comparison between measured NOEC/EC10 values from the ecotoxicity database and the proposed water quality standard calculated using the DOC correction over a range of DOC concentrations. Points indicate the test results and the line indicates the proposed water quality standard.**

Annex V of the Water Framework Directive invites a comparison of predicted EQSs with field data and to *'review the derivation to allow a more precise safety factor to be calculated'*. Such studies would ideally comprise a spectrum of species of different taxonomic groups and trophic levels, all life stages of the included organisms, realistic exposures, with replicates for each treatment, a food web including indirect effects due to competition or predation, and ecosystem function endpoints.

Recent studies of the effects of lead on molluscs (Wang et al. 2010) and mayflies in the field (Crane et al. 2007) have suggested that these organisms may be particularly sensitive to the effects of lead in aquatic exposures. *Lymnaea stagnalis* is one of the most sensitive organisms in the chronic aquatic toxicity database, by these were not tested by Wang et al (2010) and there are no reliable chronic toxicity tests on mayflies available in the dataset. Wang et al. (2010) performed acute toxicity tests on early life stages of two species of freshwater mussels, although the results of these tests, which are all greater than 100 µg $L^{-1}$, do not suggest that these are amongst the most sensitive groups of organisms for acute aquatic lead toxicity.

Crane et al. (2007) derived thresholds for dissolved lead of between 1.4 and 2.5 µg.$L^{-1}$ from field data for benthic invertebrates (527 sites sampled in 1995). The most sensitive thresholds derived were for the presence/absence of Ephemeroptera (mayflies), with slightly higher thresholds derived for the EPT metric (number of taxa from Ephemeroptera [mayflies], Plecoptera [stoneflies], or Tricoptera [caddisflies] families at a site) and for the whole community when expressed as the ASPT (average score per taxon) metric. EPT taxa are considered to be particularly sensitive to environmental stress. These thresholds were all derived using a piecewise regression technique to identify a breakpoint. Thresholds were also derived for some of the metrics of ecological quality using a quantile regression approach, which resulted in higher values (6.1 – 7.3 µg,$L^{-1}$).

In order to further assess the level of protection afforded by the proposed EQS for lead a dataset of matched UK chemical and family-level macroinvertebrate monitoring data, compiled by the Centre for Intelligent Environmental Systems (CIES, based at Staffordshire University, UK) on behalf of the Environment Agency, has been assessed with the objective of determining if "thresholds" of Pb exposure could be identified that correspond to a decline in macroinvertebrate ecological quality (as determined using the UK's RIVPACS III+ reference-based assessment tool). This dataset comprises information from standardised riverine benthic macroinvertebrate surveys taken by UK environmental agencies between 1995 and 2003 (containing presence/absence and abundance data (number of individuals per sample measured on a log scale) for 78 families across diverse taxonomic groups e.g. insects, crustaceans, molluscs, worms and leeches), associated habitat characteristics (e.g. width, depth, discharge, substrate composition) and concurrent chemical pressure monitoring data e.g. sanitary determinands, metals (including dissolved Pb), pesticides

and industrial chemicals, expressed as a median concentration for the 3 years preceding the ecological sampling. The dataset includes 1600 spring samples and 1598 autumn samples which were suitable for RIVPACS III+ (Clarke et al. 2003) predictions distributed across 341 and 350 monitoring sites, respectively, indicative of the range of riverine habitat types and environmental pressures that occur across the UK. Dissolved lead exposures ranged from 0.12 $\mu g.L^{-1}$ to 112.6 $\mu g.L^{-1}$. Many of the taxa incorporated in RIVPACS III+ have a pan-european distribution. Reference-based tools such as RIVPACS III+ are used under the Water Framework Directive for ecological classification.

Reference-based classification of riverine monitoring sites in the UK is expressed on the basis of "observed" to "expected" ratios (O/E). At a given monitoring site, the presence/absence or abundance of a particular species or family (or summary metric such as "number of taxa per sample", or "average score per taxa[2]") is compared to that which would be expected based on a database of unimpacted reference sites with similar habitat and physico-chemistry. Predictions of expected values for each family at a site were performed using RIVPACS III+ software. A value of O/E of one or greater indicates a site which is equivalent to a reference site for the taxon in question, and values of less than one indicate some deviation from reference conditions. In the case of relatively rare taxa, which are not expected to be found at a large proportion of sites, it is not uncommon for the O/E values to be close to 1 at high stressor exposures. This situation occurs where the taxon in question was not found at a site, and is expected to have a very low abundance at a site. As a result of this, particularly rare taxa are not included in this assessment as the absence of such taxa provides very limited information about ecological tolerance.

To investigate the existence of "thresholds" of Pb exposure, O/E data for the abundance of EPT taxa (which were identified as sensitive to Pb exposure by Crane et al. 2007) and abundance of snail and mussel taxa (which are the most sensitive in ecotoxicological dataset) were subject to quantile regression (based on the 90$^{th}$ percentile) against available lead concentrations (Scharf et al. 1998, Cade et al. 1999, Cade and Noon 2003, Crane et al. 2007). The taxa included in each of the group analysis is shown in Table 7.5.

O/E values for each of the groups (e.g. EPT taxa) were calculated from the observed abundance and the predicted abundance for the same site in a reference state using RIVPACS III+. An equal weighting was given to each of the scoring families in the calculation of O/E values for the groups (Equation 7.2).

$$O/E_{Group} = (\Sigma\ O_i + O_j + O_k,...+ 0.1) / (\Sigma\ E_i + E_j + E_k,...+ 0.1) \qquad \textbf{Eq. 7.2}$$

**Table 7.5 Taxa included in EPT and mollusc groups.**

| Group | Taxa (family) | Common Name |
|---|---|---|
| Molluscs | Neritidae, Viviparidae, Valvatidae, Hydrobiidae, Lymnaeidae, Physidae, Planorbidae | Snails |
| | Ancylidae, Unionidae, Sphaeriidae | Limpets and Mussels |
| EPT | Siphlonuridae, Baetidae, Heptageniidae, Leptophlebiidae, Ephemerellidae, Potamanthidae, Ephemeridae, Caenidae | Mayflies |
| | Taeniopterygidae, Nemouridae, Leuctridae, Capniidae, Perlodidae, Perlidae, Chloroperlidae | Stoneflies |
| | Rhyacophilidae, Philopotamidae, Polycentropidae, Psychomyiidae, Hydropsychidae, Hydroptilidae, Phryganeidae, Limnephilidae, Molannidae, Beraeidae, Odontoceridae Leptoceridae, Goeridae, Lepidostomatidae, Brachycentridae, Sericostomatidae | Caddisflies |

Available lead concentrations at each site were calculated from dissolved lead concentrations using the BioF relationship detailed in equations 7.3 and 7.4 below.

[2] Under the RIVPACS assessment system individual taxa are assigned "scores" based on their relative sensitivity to pollution. Higher scoring taxa are considered as relatively more sensitive to pollution than lower scoring taxa and their absence from a sample, where they are predicted to occur, is indicative of an adverse effect.

$BioF = EQS_{Reference} / EQS_{Site\ Specific}$ **Eq. 7.3**

"Available lead" = Dissolved lead . BioF **Eq. 7.4**

Available lead concentrations were calculated from DOC concentrations from catchment monitoring or estimated from dissolved iron concentrations. Catchment DOC monitoring data, expressed as the 25th percentile of available data (Environment Agency 2010b) was used preferentially. Slightly fewer than half of the samples (44%) used catchment monitoring data, and the remainder used estimation from dissolved iron concentrations for DOC concentrations.

Statistically significant quantile regressions could not be derived for all of the groups of taxa in each season. However, a decline in the 90th quantile of $O/E_{group}$ based on abundance was assessed for several groups of taxa in relation to increasing available lead exposures. EC10 values associated with this decline in abundance were derived for statistically significant models along with 95% confidence intervals (by bootstrapping using 2000 resamples). Results are given in Table 7.6

**Table 7.6 EC10 values (µg.L$^{-1}$ available lead) for groups of taxa in the spring and autumn (95% confidence interval in parenthesis)**

| Group | Spring | *p* | Autumn | *p* |
|---|---|---|---|---|
| Molluscs | 1.5 (1.0 to 1.7) | 0.042 | 1.1 (0.7 to 1.4) | 0.049 |
| Snails | 1.5 (0.8 to 3.5) | 0.050 | -- | 0.103 |
| Mussels | -- | 0.350 | 2.0 (1.1 to 3.1) | 0.007 |
| EPT | 7.6 (5.3 to 18.2) | 0.052 | -- | 0.689 |
| Mayflies | -- | 0.306 | -- | 0.752 |
| Stoneflies | -- | 0.681 | -- | 0.900 |
| Caddisflies | --- | 0.137 | -- | 0.608 |

EPT taxa would appear to be less sensitive to lead exposure than mollusc taxa. Whilst this observation is restricted to a single season it does concur with the laboratory toxicity data where molluscs are the most sensitive taxa. Comparable (within the same order of magnitude) EC10 values for molluscs are derived from the field data to those reported for *Lymnaea stagnalis* in the laboratory.

If the field evidence is used to support the derivation of the PNEC from laboratory data then the lowest EC10 of 1.1 µg l$^{-1}$ available lead for molluscs in the autumn is close to the proposed PNEC of 1.18 µg l$^{-1}$, although this threshold represents only a single season so should not be compared directly to an annual average EQS. The average of the spring and autumn analyses for molluscs is 1.3 µg l$^{-1}$ available lead. Neither of the subgroups of snails or mussels showed a similar level of sensitivity. These organisms represent the most sensitive groups of organisms from the laboratory ecotoxicity testing and other field analyses and do not suggest that there is any need for an assessment factor larger than two. The field thresholds are also derived in the presence of other pressures so should reflect to potential toxicity of lead in the presence of mixtures of other contaminants, which is likely to derive more stringent thresholds than exposure based solely on lead.

The analysis of field data for molluscs includes 10 taxa which may be expected to be sensitive to lead, including 8 taxa which are not represented in any chronic ecotoxicity testing. The analyses of EPT taxa include an additional 31 families of aquatic insects which are not represented by any chronic ecotoxicity testing. All of these groups of organisms appear to be adequately protected by the proposed standard, suggesting that the assessment factor selected is likely to be sufficient to ensure protection of sensitive organisms in the field.

## Marine

## Acute toxicity

See the earlier section on the acute toxicity of Pb for a combined freshwater and saltwater dataset.

## Chronic toxicity

The influence of abiotic factors, including DOC, on the bioavailability and toxicity of lead to saltwater species is currently unclear, and may not be comparable to the freshwater environment. As such, a conventional approach to QS derivation for the marine environment (i.e. without consideration of bioavailability) was undertaken. The *a priori* assumption in the derivation of metal EQS is that freshwater and marine datasets should not be combined. However, as no bioavailability correction for the marine EQS is proposed and there are only 9 chronic EC10/NOEC values for marine species from five taxa (EQS Guidance specifies a minimum of 10 NOECs across eight taxa for SSD derivation of HC5-50), the EQS Guidance allows the freshwater and marine datasets to be combined, unless a statistically significant difference can be observed between them. No significant difference ($p>0.05$) between mean EC10/NOEC values in the freshwater and marine datasets was detected using a t-test (equal variance) after log transformation and tests for equal variance (F-test $p>0.05$). As such, a marine EQS will be derived using combined freshwater and marine data. Because no bioavailability correction was being proposed for the marine chronic QS, no pre-selection of NOEC/EC10 values for "reasonable worst case" was undertaken and geometric species mean NOEC/EC10s were calculated as necessary from the available dataset.

An overview of the geometric mean values for the most sensitive endpoints in the combined freshwater and marine datasets is given in Table 7.

**Table 7.7 Geometric mean values of combined freshwater and marine toxicity data**

| Taxonomic group | Habitat | Species Name | NOEC/$EC_{10}$ (µg Pb $L^{-1}$ dissolved) |
|---|---|---|---|
| Algae | FW | *Pseudokirchneriella subcapitata* | 15.25 |
| | SW | *Skeletonema costatum* | 52.9 |
| | | *Dunaliella tertiolecta* | 1231.8 |
| Annelid (Polychaete) | SW | *Neanthes arenaceodentata* | 95.9 |
| Crustacean | FW | *Ceriodaphnia dubia* | 42.2 |
| | | *Hyalella azteca* | 8.2 |
| Echinoderm | SW | *Strongylocentrotus purpuratus* | 111.2 |
| | | *Dendraster excentricus* | 249.8 |
| Fish (cyprinid) | SW | *Cyprinodon variegatus* | 229.6 |
| | FW | *Pimephales promelas* | 109.46 |
| Fish (salmonid) | FW | *Salvelinus fontinalis* | 39.4 |
| Insect | FW | *Chironomus tentans* | 109.0 |
| Macrophytes | FW | *Lemna minor* | 572.79 |
| Molluscs | SW | *Crassostrea gigas* | 930.8 |
| | | *Mytilus galloprovincialis* | 51.1 |
| | | *Mytilus trossulus* | 9.2 |
| | FW | *Lymnaea stagnalis* | 1.7 |
| Rotifer | FW | *Brachionus calyciflorus* | 89.5 |
| | | *Philodina rapida* | 9.89 |
| | | | |
| **Number of taxa** | | **10** | |

| | | |
|---|---|---|
| **Number of EC10/NOEC** | **19** | |

*Generation of species sensitivity distribution and $HC_{5\text{-}50}$ calculation*

As there are sufficient data available in the combined freshwater and marine dataset (criteria for 10 NOEC/$EC_{10}$ values across a minimum of eight taxonomic groups, including specific marine taxa are met), a statistical approach (SSD) was used to derive a 5$^{th}$ percentile Hazardous Concentration ($HC_5$).

Data were analysed using RIVM ETX 2.0 (http://www.rivm.nl/rvs/risbeoor/Modellen/ETX.jsp) SSDs. Figure 7.8 shows the graphical output from the ETX lognormal model fitted to the data. The $HC_{5\text{-}50}$ is 3.79 µg.$L^{-1}$ (confidence interval (90%) = 1.05 – 9.03 µg.$L^{-1}$). All statistical tests for goodness-of-fit (Anderson-Darling, Kolmogorov-Smirnov and Cramer von Mises) were met.

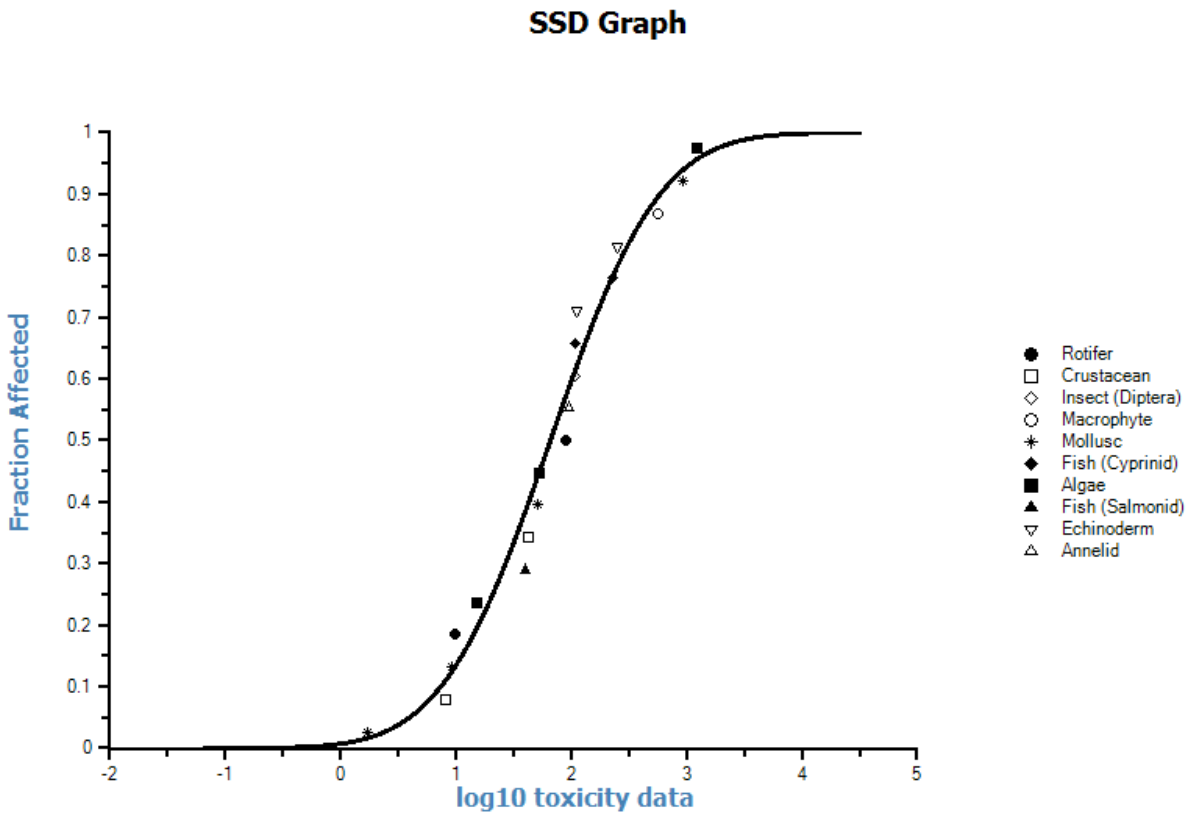


**Figure 7.8 Species Sensitivity Distribution of the species mean NOEC or $EC_{10}$ values from the combined freshwater and marine chronic Pb toxicity datasets**

As the combined freshwater and marine datasets comprises data for at least two specific marine species (echinoderms, molluscs and polycheate annelid worms) the EQS Guidance suggests an assessment factor of between 1 and 5 to be applied to the HC5-50 for EQS derivation, to be judged on a case by case basis.

Based on the available data, the following points have to be considered when determining the size of the assessment factor:

1. **The overall quality of the database and the end-points covered,** e.g. if all the data are generated from "true" chronic studies (e.g. covering all sensitive life stages);

a. The Pb database covers ecologically relevant endpoints. The selected endpoints were mortality, growth, emergence, reproduction and abnormalities;
b. The NOEC/L(E)$C_{10}$ data were extracted from tests performed in a variety of natural/artificial marine waters, covering a considerable part of the wide range of the marine water characteristics (pH value and salinity) that are normally found in European coastal waters. Ranges of pH and salinity used in the ecotoxicological tests varied respectively between 7.8-8.2 and 28-33 ppt. Therefore the Pb data properly reflect European coastal waters.
c. Coverage of sensitive life stages and chronic exposure times is also achieved for all trophic levels in the Pb database. For algae, exposure times of 3 days are available, covering different generation times. Very sensitive life stages of invertebrates are included in the database, e.g. embryos exposed for 2 to 3 days for molluscs, juvenile polychaetes exposed to Pb for 120 days. For fish very sensitive life stages are also included in the database, e.g. freshly fertilized eggs exposed to Pb for 28 days.

2. **The diversity and representativeness of the taxonomic groups covered by the database**. Chronic Pb toxicity data are available for 19 species, including nine marine species, including species form the wholly marine echinoderm and polychaete taxonomic group. The database includes broad representation of temperate marine organisms, including unicellular algae, invertebrates, and fish. No marine crustacean data were available, but data for two freshwater species (*Ceriodaphnia dubia* and *Hyalella azteca*) were available for inclusion in the database. The most sensitive data in the dataset are from the freshwater mollusc *Lymnaea stagnalis*, which has an EC10/NOEC of 1.7 µg.$L^{-1}$ dissolved Pb. The most sensitive marine species is the mollusc *Mytilus trossulus* which has an EC10/NOEC of 9.2 µg.$L^{-1}$ dissolved Pb, which is approximately five times less sensitive than *L. stagnalis*.

3. **Statistical uncertainties around the 5$^{th}$ percentile estimate,** e.g. as reflected in the goodness-of-fit or the size of the confidence interval around the 5$^{th}$ percentile:
    a. The best fitting Log-normal distribution generated a difference between the 5$^{th}$ and the 95$^{th}$ % confidence level of 1.4 – 30.5 µg.$L^{-1}$ Pb, i.e. a factor of 21.8
    b. All statistical tests for goodness-of-fit (Anderson-Darling, Kolmogorov-Smirnov and Cramer von Mises) were met

4. **Comparisons between field and mesocosm studies and the 5$^{th}$ percentile and mesocosm/field studies to evaluate the laboratory to field extrapolation.** No field data were available to allow derivation of threshold concentrations of Pb in marine waters at the field scale.

*Conclusion*

In conclusion, on the subject of the choice of the assessment factor and considering all arguments above it is felt that the most appropriate AF would be 3. Therefore, the reasonable worst case marine PNEC is proposed to be 1.3 µg dissolved Pb $L^{-1}$.

# 7.3 SEDIMENT TOXICITY

## Freshwater

According to the TGD-EQS (EC 2011) the results of long-term toxicity tests with sediment organisms are preferred for deriving sediment standards, rather than the use of acute data or freshwater ecotoxicity data in combination with an Equilibrium Partitioning approach. From the VRAR (LDAI 2008), chronic Pb toxicity data ($EC_{10}$/NOEC values) are available for seven different freshwater sediment species. These NOEC/L(E)$C_{10}$ values cover different population-related endpoints, habitats and feeding habits and are shown in Table 7.8. One of the values, for *Hexagenia limbata*, is unbounded and so has not been used in the SSD. There is no prescriptive guidance on the taxonomic requirements of datasets for the probabilistic approach taken in developing sediment EQS. That said, for Pb the VRAR and SCHER Opinion has been used as the starting point.

**Table 7.8 Species $EC_{10}$/NOEC values (total Pb) for the most sensitive endpoint for all sediment dwelling organisms**

| Organism | Most sensitive endpoint | Exposure duration | $EC_{10}$/NOEC (mg total Pb $kg^{-1}$ dry wt) |
|---|---|---|---|
| *Tubifex tubifex* | Reproduction | 28 d | 573 |
| *Ephoron virgo* | Survival | 21 d | 1,126 |
| *Hyalella azteca* | Survival | 28 d | 1,416 |
| *Gammarus pulex* | Growth | 35 d | 1,745 |
| *Lumbriculus variegatus* | Survival | 28 d | 2,100 |
| *Hexagenia limbata* | Survival, growth | 21 d | > 2,903 |
| *Chironomus tentans* | Survival | 20 d | 3,390 |

In Section 5.2.2. of the EQS Guidance there is a recommendation that, if data allow, account should be taken of bioavailability issues for metals. Specifically, for sediments this should involve consideration of the binding of organic carbon, co-precipitation and sorption by hydrous oxides or iron and manganese, and the formation of stable complexes with sulphides. The Simultaneously Extracted Metal/Acid Volatile Sulphide (SEM/AVS) approach was considered in the VRAR and the Pb effects concentrations in the sediments were modified to reflect the relative change in bioavailability. However, while the SCHER Opinion (2009) agrees that Pb sediment bioavailability should be accounted for, it did not agree with the methodology taken. This was primarily due to the limitations in the dataset in regard to the estimation of the bioavailable PNEC.

Therefore, we offer two approaches to the derivation of a sediment EQS for Pb, one using the species sensitivity distribution based on total Pb data, and the other closely following the recommendation of SCHER in the use of an assessment factor on the lowest unbounded bioavailable NOEC. It should be noted that both result in an $EQS_{sediment}$ for Pb with a relatively high degree of uncertainty.

$EQS_{sediment}$ based on the SSD approach

In this approach we evaluate the toxicity data expressed as total Pb (mg.Pb $kg^{-1}$ dry wt.) using the statistical extrapolation method applied to the chronic NOEC data for total lead shown in Table 7.8. The $HC_{5\text{-}50}$ of the best fitting species sensitivity distribution (Figure 7.9) has been calculated with the software package @RISK (Palisade Inc.)

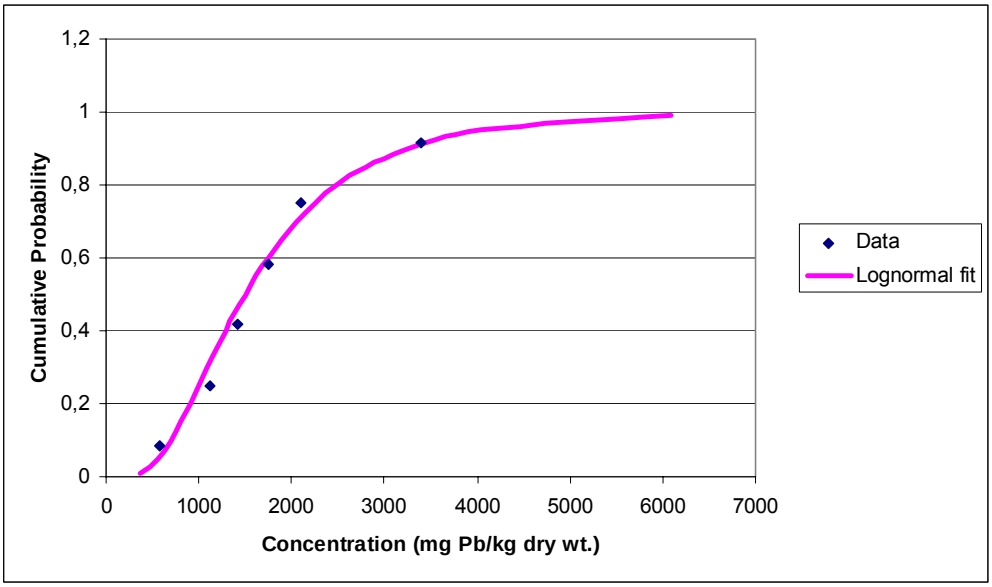


**Figure 7.9 The cumulative frequency distributions of the NOEC values (expressed as mg Pb $kg^{-1}$ dry wt.) from the Pb chronic toxicity tests towards sediment-dwelling organisms**

A summary of the estimated $HC_5$ value (with the 90% confidence bounds) for the log-normal function is provided in Table 7.9.

**Table 7.9 Calculated $HC_5$ value (mg Pb $kg^{-1}$ dry wt.) (with the 90% confidence bounds)**

| $HC_5$ at 50% (with 90% confidence bounds) expressed as mg Pb $kg^{-1}$ dry wt. | Type of best fitting model | Parameters |
|---|---|---|
| 522 (160.8-885.3) | Log-normal model | (3.18;0.261) |

The chronic sediment database for lead is relatively limited, but does contain data covering ecologically relevant endpoints for potential effects at the population level. Crucially, there do not appear to be any values below the $HC_5$.. Based on the available information, particularly the limited availability of field and mesocosm data for the sediment effects of Pb, an assessment factor of 4 should be used on the $HC_{5\text{-}50}$ value, resulting in an EQS value, without bioavailability correction, of 131 mg Pb $kg^{-1}$ dry wt. The median value for Pb concentrations in sediments from FOREGS is 14 mg $kg^{-1}$ (http://www.gtk.fi/publ/foregsatlas/maps/StreamSed/s_aricpaes_pb_edit.pdf).

$EQS_{sediment}$ taking bioavailability into account

The second approach derives an $EQS_{sediment}$ for Pb which accounts for (bio)availability using the SEM/AVS approach, modified according to the recommendation from the SCHER Opinion on the Pb VRAR. This approach uses the classical deterministic approach by applying a safety factor of 10 to the lowest unbounded bioavailable NOEC (i.e. Pb in excess of available AVS). In this case the lowest NOEC was 2.0 µmol excess Pb $g^{-1}$ dry wt. This results in a bioavailable PNEC of 0.2 µmol excess Pb $g^{-1}$ dry wt or 41 mg Pb $kg^{-1}$ dry wt.

## Saltwater

Turning to sediment EQSs for the marine compartment, there are limited chronic Pb marine data for sediments (Table 7.10) and the VRAR recommended the pooling of the marine and freshwater sediment data, which is in accordance with the recent EQS Guidance if *no documented differences existing between marine and freshwater sediment species*.

**Table 7.10 Species $EC_{10}$/NOEC values (total Pb) for the most sensitive endpoint for all sediment dwelling organisms.**

| Organism | Most sensitive endpoint | Exposure duration | AVS ($mmol.kg^{-1}$) | $EC_{10}$/NOEC (mg total $Pb.kg^{-1}$ dry wt) |
|---|---|---|---|---|
| *Neanthes arenaceodentata* | Growth | 28 d | 12.5 | 680 |
| *Leptocheirus plumulosus* | Growth | 28 d | 12.5 | 1291 |

If the long term marine and freshwater sediments effects data are pooled, a sediment effects dataset of nine $EC_{10}$/NOEC values for nine different species is available for lead, although one of these data points is unbounded. An SSD approach is taken with the toxicity data expressed as total Pb (mg Pb $kg^{-1}$ dry wt.). Using the statistical extrapolation method and the ETX program, the $HC_{5\text{-}50}$ of the conventionally used log-normal distribution was calculated (Figure 7.10).

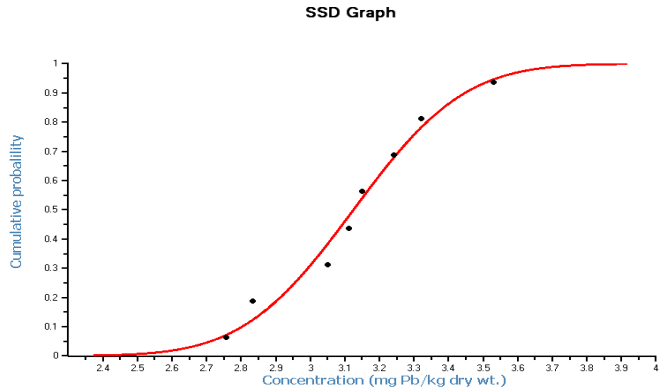


**Figure 7.10 The cumulative frequency distributions of the NOEC values (expressed as mg Pb $kg^{-1}$ dry wt.) from the Pb chronic toxicity tests on sediment-dwelling organisms**

A summary of the estimated $HC_5$ value (with the 90% confidence bounds) for the log-normal function (calculated with ETX) is provided in Table 7.11

**Table 7.11 Calculated $HC_5$ value (mg Pb $kg^{-1}$ dry wt.) (with the 90% confidence bounds)**

| $HC_5$ at 50% (& 90% confidence bounds) expressed as mg Pb kg $dry^{-1}$ wt. | Type of best fitting model | Parameters |
|---|---|---|
| 492.5 (210-765) | Log-normal model | (3.1248;0.2516) |

If similar logic is applied to the assessment factor selection for the $HC_5$ for the pooled marine SSD as applied to the freshwater SSD then the resulting $EQS_{sedimentmarine}$ would be 123 mg Pb $kg^{-1}$,.

## 7.4 SECONDARY POISONING

The VRAR (LDAI 2008) clearly demonstrated that a traditional approach, using existing guidance, for the consideration of the secondary poisoning risks of Pb was unsuitable. The result of the approach was that ambient background concentrations of Pb were predicted to present risks. The SCHER Opinion was to continue the development of a suggested dose/response assessment based on internal dose, using lead concentrations in blood for expressing that internal dose.

Wildlife biomonitoring of Pb in blood (Pb-B) has been proposed as an alternative to indirect estimates of secondary poisoning. This would be based on (new) ambient biomonitoring data of Pb-Blood for mammals and birds in Europe which are compared with critical Pb-Blood values for mammals and birds derived from the literature.

An update and refinement of the generic assessment of risk of Pb to wildlife is presented here (LDAI, 2008). Hazard assessment is based on exposure concentrations in food but this is supported by information on internal dose using concentrations of lead in blood (Buekers et al. 2008).

In the VRAR (LDAI, 2008) 20 feeding studies were selected: 8 studies were performed with mammals (6 bounded NOECs) and 12 with birds (7 bounded NOECs). An update of that database was made with 6 studies for mammals (4 bounded NOECs) and 2 studies for birds (1 bounded NOEC). The quality screening procedure was followed according to the reliability criteria from the VRAR (LDAI, 2008):

- only sub-chronic and chronic studies are included (≥ 21 days); acute studies are excluded;
- the endpoint is ecologically relevant (e.g. growth, reproduction) and not merely a biomarker for Pb exposure;
- if low doses of Pb were added to the diet (≤ 10 mg Pb $kg^{-1}$), or if NOEC ≤ 10 mg Pb $kg^{-1}$, the Pb concentration in the diet of the control animals ($C_b$) has to be measured and quality control of these measurements has to be reported;
- at least two Pb concentrations above the control have to be applied;
- mixed metal feeding studies are excluded;
- studies where Pb was injected in test animals are excluded;
- tests where Pb was administered through drinking water are excluded; and
- if multiple endpoints were used, only the lowest value was retained.

**Toxicity to birds**

An overview of the avian toxicity data from laboratory feeding studies is presented in Table 7.7.12.

**Table 7.12 Avian toxicity data from laboratory feeding studies. Concentrations are expressed per unit fresh weight of food (NOEC) or as dose rate (NOEL). DFI= daily food intake; BW= body weight.**

| Test substance | Organism | Medium | Duration | Endpoint | NOEC (mg Pb $kg_{ww}^{-1}$) | DFI/BW ($kg_{ww}.day^{-1}/kg_{bw}$)* | NOEL (mg.kg $bw^{-1}.day^{-1}$) # | LOEC (mg Pb $kg^{-1}$) (% inhibition) | LOEL (mg.kg $bw^{-1}.day^{-1}$) | Reference |
|---|---|---|---|---|---|---|---|---|---|---|
| PbOAc | 1 day old Arbor Acre broiler chicks<br><br>*Gallus domesticus* | basal diet: glucose, soybean, cottonseed oil, vitamins, minerals, NaCl, defluorinated rock phosphate, limestone, DL-methionine, nonnutrive bulk | 21-d | growth (bw) | 750 | 0.125[EU] | 93.8 [E] | 1000 | 125 | (Donaldson and Leeming 1984) |
| PbOAc | Peterson x Arbor Acre White Plymouth Rock | starter diet | 4-w | growth (bw) | 100 | 0.125[EU] | 12.5 [E] | 1000 | 125 | (Damron et al. 1969) |
| | | | | feed:gain ratio | 100 | 0.125[EU] | 12.5 [E] | 1000 | 125 | |

| Test substance | Organism | Medium | Duration | Endpoint | NOEC (mg Pb $kg_{ww}^{-1}$) | DFI/BW ($kg_{ww}.day^{-1}/kg_{bw}$)* | NOEL (mg.kg $bw^{-1}.day^{-1}$) # | LOEC (mg Pb $kg^{-1}$) (% inhibition) | LOEL (mg.kg $bw^{-1}.day^{-1}$) | Reference |
|---|---|---|---|---|---|---|---|---|---|---|
| | *Gallus sp.* | | | feed intake | 100 | 0.125 EU | 12.5 E | 1000 | 125 | |
| PbOAc | Hy-Line W-36 hens<br>*Gallus sp.* | layer ration | 10-w | growth (bw) | 200 | 0.071 D | 14.3 M | 400 | 28.5 | (Edens and Garlich 1983) |
| PbO | Warren laying hens<br>*Gallus sp.* | commercial corn-soybean meal | 75-d | feed intake | ≥ 100 | 0.125 EU | ≥ 12.5 E | | | (Meluzzi et al. 1996) |
| | | | | egg production | ≥ 100 | 0.125 EU | ≥ 12.5 E | | | |
| | | | | egg weight | ≥ 100 | 0.125 EU | ≥ 12.5 E | | | |
| | | | | shell weight | ≥ 100 | 0.125 EU | ≥ 12.5 E | | | |
| PbOAc | bobwhite quail<br>*Colinus virginianus* | basal diet: yellow corn meal, soybean meal, fish meal, alfalfa meal, animal fat, defluorinated phosphate, ground limestone, iodized salt | 6-w | growth (bw) | 2000 | 0.078 EPA | 156 E | 3000 | 234 | (Damron and Wilson 1975) |
| | | | | feed intake | 2000 | 0.078 EPA | 156 E | 3000 | 234 | |
| PbOAc | Japanese quail (F) | ground breeder ration | 32-d | liver/body weight | 250 | 0.078 EPA | 19.4 E | 500 (16%) | 38.9 | (Stone and Soares 1976) |
| PbOAc | 6 day old Japanese quail | quail starter diet | 6-w | growth (bw) | 100 | 0.078 EPA | 7.78 E | 1000 | 78 | (Morgan et al. 1975) |
| | | | | haemoglobine content | 100 | 0.078 EPA | 7.78 E | 1000 | 78 | |
| | | | 5-w | growth (bw) | 100 | 0.078 EPA | 7.78 E | 500 | 39 | |
| | | | | haemoglobine content | 100 | 0.078 EPA | 7.78 E | 500 | 39 | |
| PbOAc | Japanese quail (F) | 6 weeks complete starter-grower ration + 6 weeks complete layer ration | 12-w | growth (bw) | 100 | 0.149 D | 14.9 M | 1000 (18%) | 149 | (Edens 1985) |

** D=obtained from paper/EU=TGD-EQS/EPA=[3]; # M=calculated from measured values, E=estimated from guidance values; $ T= total concentration/ A= added concentration;*

*The daily food intake factor was derived from the data available in the article itself as long as this data (food ingestion rate and body weight) were available (D). If these data were not available, data were used from 'representative animals', obtained from annex VII of the TGD-EQS (EU) or from the data in the wildlife risk assessment handbook [3]. This resulted in calculated NOEL values (M) and estimated NOEL values (E).*

### Toxicity to mammals

An overview of the mammalian toxicity data from laboratory feeding studies is presented in Table 7.13.

**Table 7.13 Mammalian toxicity data from laboratory feeding studies. Concentrations are expressed per unit fresh weight of food (NOEC) or as dose rate (NOEL). DFI= daily food intake; BW= body weight.**

| Test substance | Organism | Medium | Duration | Endpoint | NOEC (mg Pb.$kg_{ww}^{-1}$) | DFI/BW ($kg_{ww}$/day/$kg_{bw}$)* | NOEL (mg Pb/kg bw/day) # | LOEC (mg Pb $kg^{-1}$) (% inhibition) | LOEL (mg.kg $bw^{-1}$.$day^{-1}$) | Reference |
|---|---|---|---|---|---|---|---|---|---|---|
| PbOAc | Osborne-Mendel Rat | Special diet | 333 d (parent) +90 d (offspring) | growth (bw) of P1 | ≥ 512 | 0.04[D] | ≥ 21.6[M] | | | (Morris et al. 1938) |
| | *Rattus sp.* | | | weight of offspring (F1) | 64 | 0.04[D] | 2.6[M] | 512 | 21.0 | |
| PbOAc | Wistar rat | Standard diet, 2/3 whole wheat flour, 1/3 whole milk powder +0.5 % NaCl + 0.5% $CaCO_3$ +vegetables twice a week | 10 wk | growth (bw) | 1000 | 0.07[EU] | 66[E] | 10000 (65% (M), 82 % (F)) | 660 | (Vanesch et al. 1962) |
| | *Rattus sp.* | | | | | | | | | |
| PbOAc | 45-50 g Sprague-Dawley rat (M) | semi-purified diet: casein, corn oil, cellulose, vitamins, minerals, glucose | 23-d | heme content | 150 | 0.17[D] | 25.5[M] | 300 | 51 | (Kao and Forbes 1973) |
| | *Rattus sp.* | | | | | | | | | |
| PbOAc | Wistar rat | powered laboratory animal diet with a cane sugar extracted sugar compound | 3 wk | weight of offspring | 1600 | 0.1[EU] | 160[E] | 3200 | 320 | (Mykkanen et al. 1980) |
| | *Rattus sp.* | | | | | | | | | |
| | Hooded rat | powered laboratory animal diet with a cane sugar extracted sugar compound | 3 wk | weight of offspring | ≥ 12800 | 0.1[EU] | ≥ 1280[E] | | | |
| | *Rattus sp.* | | | | | | | | | |
| PbCO3 | Long-Evans hooded rats (dams) | Ground laboratory chow | 25 d | weight of offspring | 3100 | 0.1[EU] | 155[E] | 31000 | 1550 | (Alfano and Petit 1982) |
| | *Rattus norvegicus* | | | | | | | | | |
| PbOAc | *Wistar rat (48-71 g)* | NIH-07 (cereal based diet) | 8-w | growth (bw) | 11000 | - | 854[M] | 22000 (13%) | 1591 | (Walsh and Ryden 1984) |
| | *Rattus sp.* | | | | | | | | | |
| PbOAc | male Fischer rat (31 d old) | purified AIN-76A complete meal | 44-d | growth (bw) | ≥ 127 | 0.01[D] | ≥ 1.57[M] | | | (Freeman et al. 1996) |
| | *Rattus sp.* | | | food consumption | ≥ 127 | 0.01[D] | ≥ 1.57[M] | | | |
| PbOAc | male & female Sprague-Dawley rats | AIN-76A complete meal | 30-d | growth (bw) | ≥ 250 | - | ≥ 21.2[M] | | | (Polák et al. 1996) |
| | *Rattus sp.* | | | | | | | | | |

| Test substance | Organism | Medium | Duration | Endpoint | NOEC (mg Pb.$kg_{ww}^{-1}$) | DFI/BW ($kg_{ww}$/day/$kg_{bw}$)* | NOEL (mg Pb/kg bw/day) # | LOEC (mg Pb $kg^{-1}$) (% inhibition) | LOEL (mg.kg $bw^{-1}$.$day^{-1}$) | Reference |
|---|---|---|---|---|---|---|---|---|---|---|
| $PbCO_3$ | Swiss-Webster albino mouse: adult (F) | experimental diet | 30-d | growth (bw) | 5000 | 0.12 EU | 602 E | 10000 (15%) | 1205 | (Maker et al. 1973) |
| | litter | | 60-d | brain weight | 1600 | 0.12 EU | 193 E | 4000 | 482 | |
| | C57Black/6J mouse: adult | experimental diet | 30-d | growth (bw) | 5000 | 0.12 EU | 602 E | 8000 | 964 | |
| | litter | | 60-d | brain weight | 1600 | 0.12 EU | 193 E | 4000 | 482 | |
| PbOAc | Balb/$c^{+}$ mice | | 16-m | body weight (bw) | 550 | 0.12 EU | 66.3 E | 2250 | 271 | (Eyden et al. 1978) |
| | | | | spermatozoan abnormalities | 550 | 0.12 EU | 66.3 E | 2250 | 271 | |

** D=obtained from paper/EU=TGD-EQS/EPA= [3]; # M=calculated from measured values, E=estimated from guidance values; $ T= total concentration/ A= added concentration;*

*The daily food intake factor was derived from the data available in the article itself as long as this data (food ingestion rate and body weight) were available (D). If these data were not available, data were used from 'representative animals', obtained from annex VII of the TGD (EU) or from the data in the wildlife risk assessment handbook [3]. This resulted in calculated NOEL values (M) and estimated NOEL values (E).*

### Calculation of $PNEC_{oral}$ (secondary poisoning)

The lowest NOEC for mammals is for a full chronic study for mammals (Morris et al. 1938), which resulted in a NOEC of 64 mg Pb $kg^{-1}_{ww}$ which is lower than the NOEC of 150 mg Pb $kg^{-1}_{ww}$ in the VRAR (Kao and Forbes 1973). For birds the value of 100 mg Pb $kg^{-1}_{ww}$ was used to derive the PNEC, which was observed in several experiments with Japanese quails.

The $PNEC_{oral}$ should be calculated from the lowest $NOEC_{oral}$, using an assessment factor. The assessment factor for mammals is lower than the value used in the VRAR because full chronic data are available. For both mammals and birds an assessment factor of 30 can be used. A background concentration of 1.3 mg Pb $kg^{-1}$ food is used, as explained in the VRAR (LDAI 2008). This results in:

$PNEC_{oral} = (NOEC + C_b)/AF = (64+1.3)/30 = 2.2$ mg $kg^{-1}$ food (mammals)

$PNEC_{oral} = (NOEC + C_b)/AF = (100+1.3)/30 = 3.4$ mg $kg^{-1}$ food (birds)

The assessment factor of 30 as proposed by the TGD-EQS and REACH Guidance can further be broken down in an interspecies factor (10) and a lab-field factor (3).

A species sensitivity distribution was made with the NOEC values available for mammals and birds. The species sensitivity distribution concept accounts for the differences in species sensitivity. The 18 individual bounded $NOEC_{oral}$ values in the dataset are grouped per species and per test endpoint. The geometric mean of the latter group was calculated and the lowest NOEC per species was then selected to calculate the $HC_5$. The total $NOEC_{oral}$ was calculated from the added $NOEC_{oral}$ values by adding a dietary Pb background concentration of 1.3 mg Pb $kg^{-1}_{ww}$ (LDAI, 2008). This approach resulted in 13 values for fitting the SSD curve (Table 7.12 and Figure 7.12).

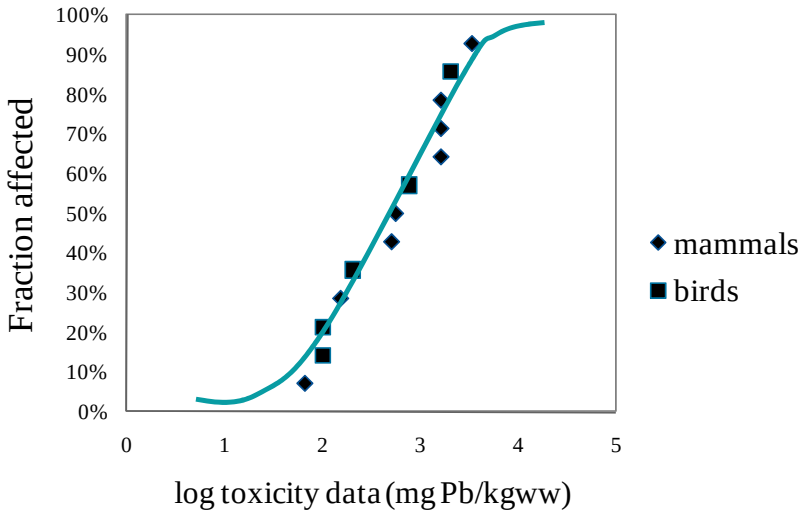


**Figure 7.12** **The cumulative frequency distribution of the selected $NOEC_{oral}$ values of Pb. Observed data and log-logistic distribution curve (best fitting curve) for the database fitted on the data.**

**Table 7.14 The chronic or sub-chronic bounded total $NOEC_{oral}$ values per species used in the statistical extrapolation method**

| Organism | Added $NOEC_{oral}$ | Cb | Total $NOEC_{oral}$ |
|---|---|---|---|
| Osborn-Mendel rat | 64 | 1.3 | 65.3 |
| Sprague-Dawley rat | 150 | 1.3 | 151.3 |
| Wistar rat | 1600 | 1.3 | 1601.3 |
| Long Evans hooded rat | 3100 | 1.3 | 3101.3 |
| Swiss webster albino mouse | 1600 | 1.3 | 1601.3 |
| C57Black/6J mouse | 1600 | 1.3 | 1601.3 |
| Balb/c+ mice | 550 | 1.3 | 551.3 |
| Holstein calves | 500 | 1.3 | 501.3 |
| Arbor Acre broiler chick | 750 | 1.3 | 751.3 |
| Arbor Acre White Plymouth Rock | 100 | 1.3 | 101.3 |
| Hy Line W36 hens | 200 | 1.3 | 201.3 |
| Bobwhite quail | 2000 | 1.3 | 2001.3 |
| Japanese quail | 100 | 1.3 | 101.3 |

Using the best fitting sensitivity distribution (ETX 2.0, (Van Vlaardingen 2004)) a total $HC_{5(oral)}$ of 52.5 mg Pb $kg^{-1}_{ww}$ (lower limit-upper limit: 14.3 - 118 mg Pb $kg_{ww}^{-1}$) could be derived. This value does not differ much from the value of 49.1 mg Pb $kg^{-1}_{ww}$, the value obtained in the VRAL (LDAI, 2008), confirming the robustness of this statistical approach.

The $HC_5$ from the SSD (52.5 mg Pb $kg^{-1}_{ww}$) is only a factor 1.2 lower than the lowest observed NOEC (64 mg Pb $kg^{-1}_{ww}$). The SSD gives a good estimate of the full range of the probable distribution of species sensitivities, including that of threatened and endangered species, especially in the case when a large database is considered. Therefore, the commonly used assessment factor of 10 for interspecies variation to derive a critical concentration from the lowest observed $NOEC_{oral}$ grossly overestimates this interspecies variation. An assessment factor of 5 is proposed to be sufficient to estimate the interspecies variation in the case of the mammalian toxicity dataset for Pb, which reduces the total assessment factor to 5x3 = 15 compared to the value of 30 recommended by the EQS Guidance for application to individual toxicity data. The use of an assessment factor of 5 is consistent with the largest assessment factor which is applied to an SSD for the aquatic or terrestrial compartments. With this new information the $PNEC_{oral}$ can be calculated as:

$PNEC_{oral} = (NOEC + C_b)/AF = (64+1.3)/15 = 4.4$ mg $kg^{-1}$ food (mammals)

$PNEC_{oral} = (NOEC + C_b)/AF = (100+1.3)/15 = 6.8$ mg $kg^{-1}$ food (birds)

$PNEC_{oral} = (HC_5 + C_b)/AF = (52.5+1.3)/15 = 3.6$ mg $kg^{-1}$ food (SSD)

$PNEC_{oral} = (NOEC + C_b)/AF = (64+1.3)/30 = 2.2$ mg $kg^{-1}$ food (mammals)

As the available data for birds and mammals do not indicate a clear difference in sensitivity between these organisms the PNEC calculated from the SSD of 3.6 mg $kg^{-1}$ food is considered to be appropriate. A study of critical blood Pb concentrations has, however, indicated that mammals may be more sensitive to Pb exposure than birds (Buekers et al. 2008).

A study of critical blood Pb concentrations (Buekers et al. 2008) in both birds and mammals has derived HC5 values separately for birds and mammals of 710 and 180 $\mu g\ l^{-1}$. The datasets upon which these HC5 values are based are considerably more extensive than that for which the HC5 presented here for food concentrations is based, and includes relevant wildlife predators such as kestrel, osprey, and vulture. The most sensitive value used in the assessment was for a rat, suggesting that a $PNEC_{oral}$ based on the chronic rat NOEC of 64 mg kg should be adequately protective of other species. More sensitive thresholds were observed in some field studies on osprey and albatross, although these were for effects on enzymes and

may not be relevant to setting an EQS. The most sensitive result, which found enzyme effects in a field study on the osprey was within a factor of 6 of the result for rats, suggesting that an assessment factor considerably lower than the default assessment factor of 30 would be sufficient to protect relevant wildlife predators from effects, even though these effects may not be relevant to EQS derivation.

An example SSD based on the dataset compiled by Beukers et al. (2008) is shown in Figure 7.13, and this clearly shows that mammals are more sensitive to Pb than birds. Data for 9 species of mammals are included, of which rats are the most sensitive. The mammal species included are rat, monkey, cow, rabbit, sheep, dog, racoon, pig, and mouse. Only the most sensitive results for each species are included in this example SSD. The bird species included are swan (2 species), duck (2 species), and kestrel. Rats are the most sensitive of 15 different species to Pb toxicity when expressed as a blood Pb concentration, suggesting that a threshold based on the rat feeding study of 64 mg $kg^{-1}$ in food is highly likely to be protective of other species, and that the use of an assessment factor of lower than 5 is likely to afford adequate protection for wildlife species.

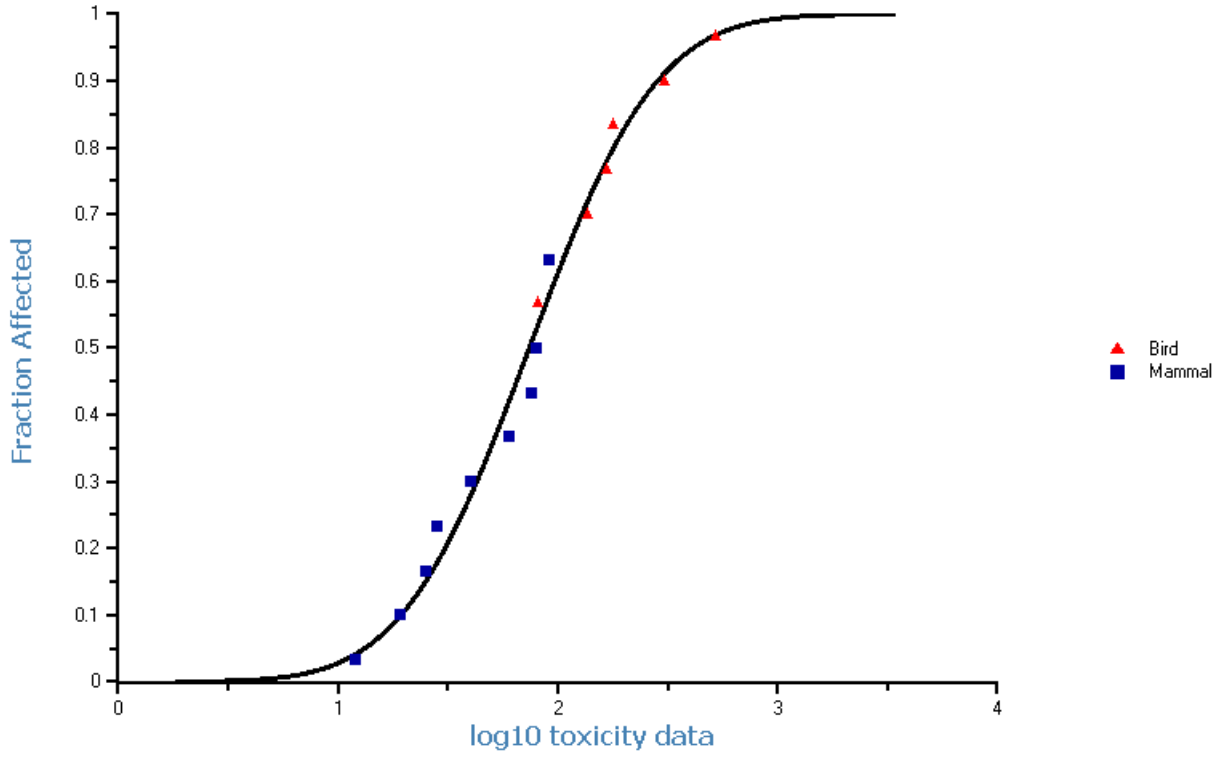


**Figure 7.13 Combined SSDD for birds and mammals based on blood Pb concentrations.**

**Conversion to water concentration**

The $PNEC_{oral}$ needs be converted into an equivalent water concentration in order to allow for a comparison against other quality standards, which are expressed as a water concentration, to be made. Bioaccumulation factors (BAF) for lead are summarised in Table 7.15. Wet weight BAF values are used in this assessment because the $PNEC_{oral}$ is also expressed as a wet weight value.

The BAF values range from 7 to 15400 $L.kg^{-1}$ (wet weight) with mean and median values of 1554 and 440 respectively. 15 out of 49 values reported are expressed as greater than values, due to the exposure concentration in the water being less than the limit of detection of <0.2 $\mu g.L^{-1}$. The values above are derived by treating these values are treated as actual values, rather than limit values. Alternatively, leaving the limit values out in the assessment results in mean and median values of 1256 and 41 respectively. The maximum and minimum values remain unchanged. Using the mean value of 1554 $L.kg^{-1}$ wwt as the BAF value a $PNEC_{water,\ secondary\ poisoning}$ of 1.42 $\mu g.L^{-1}$ can be calculated from the lowest $PNEC_{oral}$ of 2.2 mg $kg^{-1}$ (derived

from the most sensitive mammal NOEC and the default assessment factor), and a value of 2.3 $\mu g.L^{-1}$ is calculated from the selected $PNEC_{oral}$ of 3.6 $mg.kg^{-1}$ (derived from the HC5 and the revised assessment factor of 15). Both of these values are higher than the $PNEC_{aqua}$ of 1.2 $\mu g\ l^{-1}$, indicating that direct toxicity is the critical endpoint for EQS derivation.

Both of these $PNEC_{oral}$ values are considered to be conservative estimates, and the uncertainties remaining in the assessment would tend to result in higher equivalent water concentrations being calculated.

**Table 7.15 BAF values for aquatic organisms**

| Species | Organism | Tissue ($mg.kg^{-1}$ ww) | Water ($\mu g\ L^{-1}$) | BAF (ww) | Exposure media Pb analysis | Reference |
|---|---|---|---|---|---|---|
| *Asellus* | isopod | 0.688 | <0.2 | >3,440 | Filtered (0.45 µm) | Timmermans et al., 1989 |
| *Gammarus* | amphipod | 0.33 | <0.2 | >1,650 | Filtered (0.45 µm) | Timmermans et al., 1989 |
| *Cyclops* | | 0.756 | <0.2 | >3,780 | Filtered (0.45 µm) | Timmermans et al., 1989 |
| *Daphnia magna* | cladoceran | 4.6 | 3.1 | 1,500 | Filtered (0.45 µm) | Vighi, 1981 |
| *Daphnia magna* | cladoceran | 13.6 | 27.5 | 495 | Filtered (0.45 µm) | Vighi, 1981 |
| *Daphnia magna* | cladoceran | 37.4 | 13 | 2,877 | Filtered (0.45 µm) | Lu et al., 1975 |
| *Daphnia magna* | cladoceran | 30.8 | 2 | 15,400 | Filtered (0.45 µm) | Lu et al., 1975 |
| *Daphnia magna* | cladoceran | 17 | 2 | 8,500 | Filtered (0.45 µm) | Lu et al., 1975 |
| *Amblema plicata* | clam | 1.35 | 2 | 675 | Filtered (filter size not reported) | Mathis and Cummings, 1973 |
| *Dreissena* | mussel | 0.024 | <0.2 | >120 | | Timmermans et al., 1989 |
| *Dreissena polymorpha* | mussel | 0.51 | 35 | 15 | Unfiltered | Chevreuil et al., 1996 |
| *Dreissena polymorpha* | mussel | 0.37 | 54 | 7 | Unfiltered | Chevreuil et al., 1996 |
| *Dreissena polymorpha* | mussel | 0.32 | 37 | 9 | Unfiltered | Chevreuil et al., 1996 |
| *Dreissena polymorpha* | mussel | 0.19 | 12 | 16 | Unfiltered | Chevreuil et al., 1996 |
| *Dreissena polymorpha* | mussel | 0.14 | 8 | 18 | Unfiltered | Chevreuil et al., 1996 |
| *Fusconaia flava* | clam | 1.85 | 2 | 925 | Filtered (filter size not reported) | Mathis and Cummings, 1973 |
| *Lymnaea* | snail | 0.079 | <0.2 | >395 | Filtered (0.45 µm) | Timmermans et al., 1989 |
| *Potamopyrgus* | snail | 0.77 | <0.2 | >3,850 | Filtered (0.45 µm) | Timmermans et al., 1989 |
| *Quadrula quadrula* | clam | 1.1 | 2 | 550 | Filtered (filter size not reported) | Mathis and Cummings, 1973 |
| *Physa* | snail | 33.4 | 13 | 2,570 | Filtered (0.45 µm) | Lu et al., 1975 |

| Species | Organism | Tissue ($mg.kg^{-1}$ ww) | Water ($\mu g\ L^{-1}$) | BAF (ww) | Exposure media Pb analysis | Reference |
|---|---|---|---|---|---|---|
| *Physa* | snail | 8.8 | 2 | 4,400 | Filtered (0.45 µm) | Lu et al., 1975 |
| *Physa* | snail | 5.6 | 2 | 2,800 | Filtered (0.45 µm) | Lu et al., 1975 |
| *Chironomus* | midge | 0.366 | <0.2 | >1,830 | Filtered (0.45 µm) | Timmermans et al., 1989 |
| *Glyptotendipes* | midge | 0.088 | <0.2 | >440 | Filtered (0.45 µm) | Timmermans et al., 1989 |
| *Holocentropus* | caddisfly | 0.264 | <0.2 | >1,320 | Filtered (0.45 µm) | Timmermans et al., 1989 |
| *Ischnura* | damselfly | 0.35 | <0.2 | >1,750 | Filtered (0.45 µm) | Timmermans et al., 1989 |
| *Limnephilus* | caddisfly | 0.872 | <0.2 | >4,360 | Filtered (0.45 µm) | Timmermans et al., 1989 |
| *Stictochironomus* | chironomid | 1.062 | <0.2 | >5,310 | Filtered (0.45 µm) | Timmermans et al., 1989 |
| *Micronecta* | corixid | 0.374 | <0.2 | >1,870 | Filtered (0.45 µm) | Timmermans et al., 1989 |
| *Erpobdella* | leech | 0.324 | <0.2 | >1,620 | Filtered (0.45 µm) | Timmermans et al., 1989 |
| *Hygrobates* | mite | 0.346 | <0.2 | >1,730 | Filtered (0.45 µm) | Timmermans et al., 1989 |
| *Astyanax mexicanus* | fish | 0.2 | 14 | 14 | Unfiltered | Villarreal-Trevino et al., 1986 |
| *Astyanax mexicanus* | fish | 0.18 | 12 | 15 | Unfiltered | Villarreal-Trevino et al., 1986 |
| *Astyanax mexicanus* | fish | 0.172 | 10 | 17 | Unfiltered | Villarreal-Trevino et al., 1986 |
| *Astyanax mexicanus* | fish | 0.16 | 7 | 23 | Unfiltered | Villarreal-Trevino et al., 1986 |
| *Astyanax mexicanus* | fish | 0.948 | 4 | 237 | Unfiltered | Villarreal-Trevino et al., 1986 |
| *Cichlasoma cyanoguttatum* | fish | 0.1 | 9 | 11 | Unfiltered | Villarreal-Trevino et al., 1986 |
| *Cichlasoma cyanoguttatum* | fish | 0.272 | 14 | 19 | Unfiltered | Villarreal-Trevino et al., 1986 |
| *Cichlasoma cyanoguttatum* | fish | 0.26 | 10 | 26 | Unfiltered | Villarreal-Trevino et al., 1986 |
| *Micropterus salmoides* | fish | 0.092 | 9 | 10 | Unfiltered | Villarreal-Trevino et al., 1986 |
| *Notropos lutrensis* | fish | 0.16 | 14 | 11 | Unfiltered | Villarreal-Trevino et al., 1986 |
| *Poecilia reticulata* | Fish | 3.2 | 3.1 | 1,032 | Filtered (0.45 µm) | Vighi, 1981 |
| *Poecilia reticulata* | fish | 7.2 | 27.5 | 260 | Filtered (0.45 µm) | Vighi, 1981 |
| *Poecilia formosa* | fish | 0.18 | 14 | 13 | Unfiltered | Villarreal-Trevino et al., 1986 |

| Species | Organism | Tissue ($mg.kg^{-1}$ ww) | Water ($\mu g\ L^{-1}$) | BAF (ww) | Exposure media Pb analysis | Reference |
|---|---|---|---|---|---|---|
| *Poecilia formosa* | fish | 0.26 | 9 | 29 | Unfiltered | Villarreal-Trevino et al., 1986 |
| *Poecilia formosa* | Fish | 0.452 | 12 | 38 | Unfiltered | Villarreal-Trevino et al., 1986 |
| *Poecilia formosa* | Fish | 0.432 | 10 | 43 | Unfiltered | Villarreal-Trevino et al., 1986 |
| *Poecilia formosa* | Fish | 0.26 | 4 | 65 | Unfiltered | Villarreal-Trevino et al., 1986 |
| *Poecilia formosa* | Fish | 0.56 | 7 | 80 | Unfiltered | Villarreal-Trevino et al., 1986 |

Two recent reviews of metal bioaccumulation (McGeer et al. 2003; De Forest et al. 2007) are also consistent with these findings. De Forest et al. (2007) indicates only a slight tendency for variation in BAF with exposure concentrations for mussels exposed to lead, and no tendency for variation in BCF with exposure. This is consistent with the approach taken in the report regarding the effect of exposure concentration on the BAF. A median BAF of 3321 (dry weight) is reported for Pb, A wet weight BAF (as used for the assessment) would most likely be lower. Using moisture contents for organisms reported by Ikemoto et al (2008) would result in a median BAF value of less than 1000. McGeer et al. (2003) report significant negative relationships between exposure and BCF/BAF for 4 out of 8 analyses, suggesting that there is the possibility of such a relationship for some groups of organisms. BAF values are reported on a wet weight basis in this study, explaining the apparent difference between the results reported here and those reported by De Forest et al (2007). An average BAF of 350 (+/- 431) is reported, and an average BCF of 598 (+/- 1102) or 410 (+/- 647) when only data for exposure concentrations between 1 and 15 $\mu g\ l^{-1}$ are considered.

A study by Ikemoto et al (2007) reports some very high BAF values for fish and invertebrates. The study reports BAF values for crustaceans and fish in a river with very low dissolved lead concentrations of <4 and 8 $ng\ l^{-1}$. The mean BAF for all fish and crustaceans was 7,120 on a wet weight basis (using taxa specific moisture contents reported in the paper). Few details of the water analysis are provided and the authors have been contacted for further details. It may be that the very low apparent water concentrations result in unusually high BAF values being calculated. If a water concentration of 0.05 $\mu g\ l^{-1}$ is assumed (a value which would be used if the LoD is 0.1 $\mu g\ l^{-1}$, adequate for assessing compliance against the proposed EQS for water) then the mean BAF would be 1240 on a wet weight basis. The fact that the reported dissolved lead concentrations are over an order of magnitude lower than any other reported dissolved lead concentrations results in BAF values which are rather higher than other data. The maximum concentration is over an order of magnitude lower than the lowest exposure concentration considered by De Forest et al. (2007). The lack of detail regarding the analysis of water concentrations, the fact that the water concentrations are much lower than those reported for any other studies, and the influence of the water concentration on the finally calculated BAF, suggest that the BAF values form this study should be treated with caution.

Regardless of the accuracy and representivity of the reported water concentrations it is clear that dissolved lead concentrations in the river were low. Despite the low dissolved exposure concentrations some of the fish sampled in the study contained lead at levels which are above the lower acceptable threshold level for lead in fisheries products to protect human health. The thresholds set in Europe relate to fish muscle meat, and are dependent upon species. None of the species of fish contained lead at a concentration of greater than 0.4 $mg\ kg^{-1}$ (upper EU threshold for protection of human health from consumption of fish muscle meat), although some fish contained lead at concentrations of greater than 0.2 $mg\ kg^{-1}$ (lower EU threshold for protection of human health from consumption of fish muscle meat), although these were also analysed on a whole body basis, and the largest of these fish had a mean body weight of less than 30g, so may not be an important human food source. It is clear that the concentrations of lead in some species of fish can exceed 0.2 $mg\ kg^{-1}$ even when dissolved lead exposure concentrations appear to be very low. This would suggest that either the acceptable concentration of dissolved lead should be less than 5 $ng\ l^{-1}$ in order to ensure protection of humans from the consumption of fisheries products, or that factors other than the dissolved concentration in the water are more important in determining lead uptake by organisms, and that dissolved lead should not be used as an indication of whether or not bioaccumulation may occur, which is more

probable. This is consistent with the conclusion that the uncertainties surrounding the bioaccumulation of lead are very large.

A study on known metal accumulator species (Ravera et al. 2003) used unpurged mussels and also included the periostracum (a skin-like covering on the outside of the shell). The influence of the gut contents on the overall body burden was assumed to be negligible, although this may not necessarily be the case for lead. Inclusion of the periostracum in the analysis may mean that lead associated with particles which are adsorbed to the outer shell surface are also included in the analysis. It is unlikely that the BAF values reported are relevant to the consumption of fisheries products (either by humans of animals) due to the inclusion of the periostracum and gut contents, and also because accumulator species were specifically selected for the study. The BAF values reported (8,000, 17,000, and 25,000) for the soft tissues (including periostracum and gut contents) of 3 mussel taxa are likely to overestimate accumulation of lead, and may include a contribution from material which has not been absorbed by the organism.

The levels of lead in the mussels exceeded the standard for lead in mussel flesh, even though the water concentration was 10 times lower than the proposed EQS for water. It is considered that the findings of these two studies indicate that the uncertainties involved in extrapolation from a level in biota to a water concentration are substantial and that it is not possible to say with any certainty whether direct aquatic toxicity or secondary poisoning is the critical endpoint when assessed on the basis of extrapolated water concentrations. Factors other than simply the dissolved concentration of Pb in water (e.g. DOC) are likely to be more important in determining the potential for bioaccumulation.

The BAF values for lead appear to show a tendency for higher values at low exposure concentrations and lower values at higher exposure concentrations where multiple data are available for the same species. These apparent relationships between the exposure concentration and BAF are, however, not significant at the 95% confidence level in any cases, suggesting that the BAF value used to extrapolate from a $PNEC_{oral}$ to an equivalent water concentration should not be adjusted to take account of likely exposure concentrations. A similar conclusion was drawn by both McGeer et al (2003) and De Forest et al. (2007). As a result of this the mean BAF value used to calculate the equivalent water concentration is considered to be appropriate. Chronic studies on fish (Mager et al. 2004; Grosell et al 2006b) have also shown that DOC complexation reduces Pb accumulation in addition to reducing Pb toxicity. This suggests that at sites where a higher standard for direct aquatic toxicity is considered to be appropriate increased accumulation of Pb is unlikely to occur.

A critical BAF value can be calculated as the minimum BAF value which would be required for the corresponding $PNEC_{water,\ secondary\ poisoning}$ to be lower than the $PNEC_{aqua}$ for direct chronic toxicity. These values are 3051 $L.kg^{-1}$ wet weight for the $PNEC_{oral}$ of 3.6 $mg.kg^{-1}$ (derived from the HC5 and an assessment factor of 15), and a value of 3729 $L.kg^{-1}$ wet weight for the $PNEC_{oral}$ of 4.4 $mg.kg^{-1}$ (derived from the most sensitive mammal NOEC and an assessment factor of 15). These BAF values are exceeded by 16.3% and 14.3% of the 49 BAF values respectively when limit values are treated as absolute values. This suggests that circumstances where secondary poisoning could be of greater concern than direct aquatic toxicity are extremely unlikely.

The $PNEC_{oral}$ is therefore 3.6 $mg.kg^{-1}$ wet weight in food, and the corresponding $PNEC_{water,\ secondary\ poisoning}$ is 2.3 $\mu g.L^{-1}$.

| | |
|---|---|
| **$PNEC_{oral}$** | **3.6 $mg.kg^{-1}$ wet weight in food** |
| **$PNEC_{water,\ secondary\ poisoning}$** | **2.3 $\mu g.L^{-1}$** |

## 7.5 PROTECTION OF HUMANS AGAINST ADVERSE HEALTH EFFECTS FROM DRINKING WATER AND FISHERIES PRODUCTS

In accordance with the TGD-EQS (EC 2011), where there are existing standards for the protection of human health established in European legislation these shall be used for the purposes of WFD EQS derivation.

A limit for lead of 10 $\mu g.l^{-1}$ in tap water is specified in Council Directive 98/83/EC. This limit is above the $PNEC_{aqua}$ of 1.2 $\mu g\ l^{-1}$, indicating that direct toxicity is the critical endpoint for WFD EQS derivation.

European Council Regulation (EC) No 78/2005 (amending Regulation 466/2001) has established tolerable limits on lead in foodstuffs including edible aquatic species, such as fish, crustaceans, bivalve molluscs and cephalopods to protect human health.

| Human health via consumption of drinking water | Master reference |
|---|---|
| 10 µg.l$^{-1}$ (preferred regulatory standard) | Directive 98/83/EC |

| Human health via consumption of fishery products | Master reference |
|---|---|
| Fish muscle meat (dependant on species): 0.2 or 0.4 mg.kg$^{-1}_{\text{biota ww}}$<br>Crustaceans, excluding brown meat of crab: 0.5 mg.kg$^{-1}_{\text{biota ww}}$<br>Bivalve molluscs: 1.0 mg.kg$^{-1}_{\text{biota ww}}$<br>Cephalopds (excluding viscera): 1.0 mg.kg$^{-1}_{\text{biota ww}}$ | Council Regulation (EC) No 78/2005, amending Regulation 466/2001 |

The TGD-EQS invites the uncertainties involved in converting a $QS_{Biota}$ into an equivalent water-column concentration to be considered. This is done by performing the conversion for extreme BAF values, as well as typical BAF values. If the QS for water lies within the range of possible extrapolated values for of the QS biota, when considering the uncertainties of the extrapolation, it is not possible to determine with high confidence which is the critical QS. A summary of the available BAF data for lead is given in Tables 7.15 and 7.16 for all data, and filtered (dissolved) data only, respectively. BAF values are summarised for all taxa, and also for specific groups of taxa (fish, crustacea, and molluscs).

**Table 7.15 BAF data for lead**

| Data | Minimum | Median | Mean | Maximum | Number |
|---|---|---|---|---|---|
| All | 7 | 440 | 1554 | 15400 | 49 |
| Fish | 10 | 25 | 108 | 1032 | 18 |
| Crustacea | 495 | 3159 | 4705 | 15400 | 8 |
| Molluscs | 7 | 473 | 1168 | 4400 | 14 |

**Table 7.16 BAF data for lead using filtered measurements only**

| Data | Minimum | Median | Mean | Maximum | Number |
|---|---|---|---|---|---|
| All | 120 | 1740 | 2695 | 15400 | 28 |
| Fish | 260 | 646 | 646 | 1032 | 2 |
| Crustacea | 495 | 3159 | 4705 | 15400 | 8 |
| Molluscs | 120 | 925 | 1809 | 4400 | 9 |

These BAF values are used in conjunction with the various threshold values for acceptable levels of lead in fishery products (for fish, crustaceans, and molluscs) to calculate equivalent water concentrations for comparison against the PNEC for direct aquatic toxicity (1.18 µg l$^{-1}$). A summary of the maximum and minimum values derived is shown in Table 7.17.

**Table 7.17 Range of possible water concentrations required to protect against lead accumulation in fisheries products for human consumption (BAF data). Expressed as water concentrations µg l$^{-1}$.**

| BAF data used | Fish | | Crustacea | | Molluscs | |
|---|---|---|---|---|---|---|
| | Minimum | Maximum | Minimum | Maximum | Minimum | Maximum |
| Taxa specific BAF | 0.2 | 20 | 0.03 | 1.0 | 0.2 | 143 |
| Taxa specific filtered BAF | 0.2 | 0.8 | 0.03 | 1.0 | 0.2 | 8.3 |
| All BAF | 0.01 | 29 | 0.03 | 71 | 0.07 | 143 |

| | | | | | | |
|---|---|---|---|---|---|---|
| All filtered BAF | 0.1 | 1.7 | 0.03 | 4.2 | 0.07 | 8.3 |

Grey: Below FOREGS Background concentration, Green: Below PNEC for direct aquatic toxicity, Red: Above PNEC for direct aquatic toxicity

All of the lower bounds for predictions (from maximum BAF values) are within the expected background range for Pb reported in the FOREGS dataset (see below). Only the extrapolated water concentration for the crustacean standard using taxa specific data, and the fish standard using taxa specific filtered data, have ranges which are entirely below the aquatic PNEC for direct toxic effects (although only by a relatively small margin). In the case of crustacea this is likely to be due to the fact that the BAF values were derived for very small crustacean species (*asellus*, *cyclops*, *gammarus*, and *daphnia*) which are unlikely to be consumed by humans, but have high bioaccumulation factors due to their high surface area relative to their small size. In the case of fish the extrapolated water concentration when only filtered taxon specific data are used is also below the aquatic PNEC for direct toxic effects. This is considered to be due to the very small number of BAF measurements available for fish using filtered measured data.

As there are uncertainties surrounding the most appropriate selection of BAF data the calculations are also performed using BCF data. All of the calculations based on BCF bracket the QS water. Relevant BCF data are summarised in Table 7.18. The resulting range of extrapolated water concentrations which would be expected to be protective of human health from fisheries products are shown in Table 7.19.

**Table 7.18 Summary of BCF data for lead**

| Data | Minimum | Median | Mean | Maximum | Number |
|---|---|---|---|---|---|
| All | 5 | 424 | 728 | 8000 | 45 |
| Fish | 5 | 44 | 217 | 1322 | 13 |
| Crustacea | 110 | 650 | 1257 | 8000 | 11 |
| Molluscs | 110 | 354 | 598 | 2500 | 11 |

**Table 7.19 Range of possible water concentrations required to protect against lead accumulation in fisheries products for human consumption (BCF data). Expressed as water concentrations μg $l^{-1}$.**

| BCF data used | Fish | | Crustacea | | Molluscs | |
|---|---|---|---|---|---|---|
| | Minimum | Maximum | Minimum | Maximum | Minimum | Maximum |
| Taxa specific BCF | 0.2 | 40 | 0.06 | 4.5 | 0.4 | 9.0 |
| All BCF | 0.02 | 40 | 0.06 | 100 | 0.1 | 200 |

Grey: Below FOREGS Background concentration, Green: Below PNEC for direct aquatic toxicity, Red: Above PNEC for direct aquatic toxicity

Overall, in the majority of analyses, the range of possible extrapolated water PNEC concentrations include values which are both higher and lower than the proposed aquatic PNEC for direct toxic effects. It is therefore not possible to determine with confidence that the standard for the protection of human health from the consumption of fishery products is the critical quality standard. Given the limitations of the extrapolation the proposed aquatic PNEC for direct toxic effects is recommended as the critical quality standard.

The uncertainty in the level of the $QS_{Water}$ which is required to ensure protection against the established $QS_{Biota(HH)}$ occurs because of uncertainties in the bioaccumulation of lead, rather than any uncertainties in the level of the $QS_{Biota(HH)}$. The levels of lead in food derived from freshwater fisheries will continue to be monitored in accordance with existing legislation. A recent EFSA report on lead in food (EFSA 2010) reports the ranges of lead concentrations in a variety of fisheries products, and these are summarised in Table 7.20.

**Table 7.20 Ranges of lead concentrations in fisheries products reported by EFSA 2010**

| Food category | N | % <LOD | Data treatment[1] | P5 | Median | P95 |
|---|---|---|---|---|---|---|
| Bivalve molluscs | 2231 | 35.0 | LB | 0.0000 | 0.1100 | 0.7578 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | UB | 0.0200 | 0.2000 | 0.7578 |
| Cephalopods | 368 | 76.1 | LB | 0.0000 | 0.0000 | 0.1000 |
| | | | UB | 0.0080 | 0.0400 | 0.3000 |
| Crustaceans | 1580 | 79.7 | LB | 0.0000 | 0.0000 | 0.1000 |
| | | | UB | 0.0080 | 0.1700 | 0.2000 |
| Fish and fish products | 6991 | 76.8 | LB | 0.0000 | 0.0000 | 0.0800 |
| | | | UB | 0.0050 | 0.0200 | 0.2000 |

1: LB (Lower bound), all values <LOD treated as zero. UB (upper bound), all values <LOD treated as LOD.

It is clear from this information that the vast majority of fisheries products (>95%) are not contaminated with lead at levels above those which are considered to be acceptable for human consumption.

Lead concentrations at unimpacted and background sites were monitored under the FOREGS programme. Ambient background lead concentrations in surface waters range from 0.015 μg $l^{-1}$ to 0.63 μg $l^{-1}$ ($5^{th}$ and $95^{th}$ percentile values respectively). The median concentration of dissolved lead was 0.09 mg l-1, which is close to, or above, the lower estimates of acceptable water concentrations for the protection of human health from the consumption of fisheries products. This suggests that the uncertainties associated with the extrapolation from an acceptable concentration in food to an acceptable concentration in water which would be protective of the consumption of that food introduces a greater level of uncertainty than is removed by enabling a comparison between the PNEC for direct aquatic toxicity and the $PNEC_{Oral}$ for the protection of human health.

Whilst under some circumstances lead concentrations in whole organisms may exceed the standard set for the protection of human health (Ravera et al. 2003; Ikemoto et al. 2007), even when the dissolved lead exposure concentrations are well below the proposed EQS for water, the uncertainties involved in extrapolation from a level in biota to a water concentration are sufficiently great that it is not possible to say with any certainty whether direct aquatic toxicity or secondary poisoning is the critical endpoint when assessed on the basis of extrapolated water concentrations. Other factors are likely to be more important in determining the potential for bioaccumulation than the dissolved concentration in water.

It is therefore recommended that the PNEC for direct aquatic toxicity is considered as the critical PNEC value.

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# ANNEX 1

## Indicative Compliance Exercise

Any EQS regime needs to reflect the real risk to the environment and the protection goals being sought in order to avoid either unnecessary costs to society or possible environmental impacts. However, an EQS regime also needs to be as simple as possible to minimise regulatory burdens (Environment Agency 2010). The balance between precision and practicality for a metal EQS is facilitated if account is taken of metal bioavailability. Monitoring datasets from England and Wales, Austria and Sweden are used in this Annex to give an indication of the likely compliance picture for waters across a range of physicochemical characteristics and exposure conditions.

The dataset from England and Wales is for freshwaters from rivers and lakes across a range of physicochemical conditions. These data from the years 2006-2009 are representative of annual average Pb concentrations calculated according to the WFD guidance (EC 2009) and median site DOC concentrations. Although there are only 224 monitoring sites, these are representative of 2571 individual matched samples. These data were provided by the Environment Agency of England and Wales.

The Swedish dataset is for freshwaters from 2000-2008. These data have been used in an indicative face-value compliance assessment, i.e. annual averages have not been yet calculated. Effectively, this leads to a more precautionary approach as data extremes are still present in the dataset. The median DOC for these data is 8.4 $mg.L^{-1}$. These data are from EIONET.[3]

The Austrian monitoring data used in this exercise is from 2000-2004 for freshwater sites from rivers. The dissolved Pb data and measured DOC were matched for each site. Median DOC for the Austrian dataset was relatively low at 1.3 $mg.L^{-1}$. These data, like the Swedish dataset, have been used in a face-value compliance, i.e. annual averages have not been yet calculated. The values recorded as 'at or below the limit of quantitation' were treated according to the WFD guidance (EC 2009). These data were taken from the website of the Umweltbundesamt, Austria[4].

**Table A. Number of sites progressing through two tiers of the compliance assessment based on a generic $EQS_{available}$ of 1.2 µg Pb $L^{-1}$. The Tiers correspond to those shown in Figure 7.2.**

| Progression | England and Wales (n =224)* | Austria (n =6238)# | Sweden (3907) |
|---|---|---|---|
| Tier 1. Generic $EQS_{available}$ | 79 | 134 | 269 |
| Number of sites going progressing beyond Tier 2. Pb Screening tool | 27 | 107 | 2 |
| Percentage of sites being removed at Tier 2. | 88 | 98 | >99 |

The indicative compliance picture presented above suggests a high level of compliance at Tier 2, with the greatest percentage of sites (still only 12%) in England and Wales progressing to Tier 3 for local consideration of ambient background concentrations.

[3] http://www.eionet.europa.eu/

[4] http://wisa.lebensministerium.at/

# ANNEX 2

## Selection of "reasonable worst-case" chronic toxicity dataset for Pb $HC_{5-50}$ calculation

Where multiple $NOEC/EC_{10}$ values are available for a species, providing that tests were conducted according to a comparable methodology (e.g. exposure duration, endpoints, temperature), current EQS guidance allows a geometric mean of the results to be used for PNEC derivation. This annex summaries the rationale and process by which "species mean" $NOEC/EC_{10}$ values were calculated for lead PNEC derivation where a range of values are present in the dataset which were obtained from tests with different lead bioavailability.

As the bioavailability of lead is known to be influenced by test media physicochemistry (e.g. pH, hardness and dissolved organic carbon (DOC) concentrations), the data used for PNEC derivation should be obtained from tests conducted under physicochemical conditions consistent with "reasonable worst-case" lead bioavailability and toxicity. As such, tests conducted under conditions of limited lead bioavailability (either as a result of test pH, hardness or DOC, or any combination of these parameters) should be excluded from the dataset used for PNEC derivation. This is in order that the subsequently derived PNEC is sufficiently protective of the waters it is intended to protect.

To achieve this, Principal Components Analysis (PCA) was applied to each species' ecotoxicological dataset to identify a sub-set of tests that were conducted under physicochemical conditions consistent with "reasonable worst-case" lead bioavailability and toxicity. PCA is a technique for approximating high-dimensional (multivariate) information in low (usually two) dimensional plots (Everitt 1978, Chatfield and Collins 1980). "Similar" tests, in terms of their physicochemistry, are plotted closer together on an ordination than tests with "dissimilar" physicochemistry. Using this technique, in combination with the available NOEC/EC10 data, clusters of tests that have comparable physicochemistry and are consistent with "reasonable worst-case" bioavailability and toxicity can be objectively identified for subsequent use in PNEC derivation. PCA ordination was conducted on normalised matrices of Euclidian distance using Primer 6 software (version 6.1.1.3, Primer-E Ltd, http://www.primer-e.com/).

### *Ceriodaphnia dubia*

Thirty one individual $NOEC/EC_{10}$ values for chronic tests conducted with *Ceriodaphnia dubia* were available for PNEC derivation (Table 1). The geometric mean NOEC/EC10 of all the available tests was 42.2 µg dissolved Pb. $L^{-1}$. PCA Eigenvalues 1 and 2 explain 45.4 and 31.3 % of the variability in the dataset, respectively (Figure 1). The variability in DOC is nearly completely explained by PC 1 and 2. The influence of DOC, hardness and pH on the resulting clustering of tests can be interpreted from Figure 2. A cluster of tests characterised by low DOC and low hardness is apparent at the bottom right of the ordination. A geometric mean $NOEC/EC_{10}$ of the cluster of 12 "similar" tests consistent with "reasonable worst case" bioavailability is 36.8 µg dissolved Pb. $L^{-1}$.

**Table 1 Complete chronic *C. dubia* dataset of tests conducted under comparable methodology. "Reasonable worst-case" sub-set identified in bold.**

| CSR Test ID | $NOEC/EC_{10}$ ($\mu g.L^{-1}$) | DOC ($mg.L^{-1}$ C) | pH | Hardness ($mg.L^{-1}$) | Reference |
|---|---|---|---|---|---|
| **33** | **38.9** | **2.2** | **7.2** | **35** | Grosell, 2010a |
| **34** | **38.0** | **1.2** | **7.6** | **52** | Grosell, 2010a |
| 35 | 12.4 | 1.2 | 7.3 | 172 | Grosell, 2010a |
| 36 | 8.4 | 1.2 | 7.5 | 362 | Grosell, 2010a |

| CSR Test ID | NOEC/$EC_{10}$ ($\mu g.L^{-1}$) | DOC ($mg.L^{-1}$ C) | pH | Hardness ($mg.L^{-1}$) | Reference |
|---|---|---|---|---|---|
| 37 | 19.6 | 1.2 | 7.5 | 524 | Grosell, 2010a |
| **38** | **51.7** | **2.5** | **7.5** | **30** | Grosell, 2010a |
| 39 | 150.0 | 5.4 | 7.4 | 30 | Grosell, 2010a |
| **40** | **100.6** | **2** | **7.4** | **22** | Grosell, 2010a |
| **41** | **88.5** | **2.5** | **7.3** | **26** | Grosell, 2010a |
| 42 | 44.1 | 4.9 | 7.4 | 24 | Grosell, 2010a |
| 43 | 247.9 | 7.2 | 7.3 | 37 | Grosell, 2010a |
| 44 | 21.4 | 1.2 | 7.4 | 135 | Grosell, 2010a |
| **45** | **19.8** | **1.2** | **7.1** | **26** | Grosell, 2010a |
| **46** | **16.6** | **1.2** | **7.2** | **24** | Grosell, 2010a |
| **47** | **46.2** | **1.2** | **7.7** | **25** | Grosell, 2010a |
| 48 | 48.6 | 1.2 | 7.9 | 26 | Grosell, 2010a |
| 49 | 94.0 | 1.2 | 8.2 | 26 | Grosell, 2010a |
| **50** | **13.8** | **1.2** | **7.3** | **23** | Grosell, 2010a |
| **53** | **36.0** | **0.7** | **7.55** | **40** | Parametrix, 2009 |
| 54 | 31.4 | 0.5 | 8.15 | 134 | Parametrix, 2009 |
| 55 | 130.0 | 0.5 | 8.25 | 238 | Parametrix, 2009 |
| 56 | 48.1 | 0.5 | 8.1 | 290 | Parametrix, 2009 |
| **61** | **33.3** | **0.5** | **6.05** | **52** | Parametrix, 2009 |
| **62** | **35.9** | **0.5** | **7** | **52** | Parametrix, 2009 |
| 63 | 20.4 | 0.51 | 8 | 52 | Parametrix, 2009 |
| 64 | 58.8 | 0.5 | 8.5 | 52 | Parametrix, 2009 |
| 69 | 30.2 | 2.7 | 7.9 | 32.5 | Grosell, 2010b |
| 71 | 107.4 | 9.6 | 8.47 | 223.4 | Grosell, 2010b |
| 72 | 354.9 | 17.3 | 7.31 | 27.5 | Grosell, 2010b |
| 73 | 7.6 | 8.2 | 7.07 | 16.3 | Grosell, 2010b |
| 74 | 57.4 | 1.7 | 8.33 | 246.9 | Grosell, 2010b |
| Summary based on “all data” | | | | | |
| **Min** | 7.6 | 0.5 | 6.1 | 16.3 | |
| **Max** | 354.9 | 17.3 | 8.5 | 524.0 | |
| **Geomean** | 42.2 | 1.6 | 7.6 | 56.4 | |
| Summary based on “Reasonable worst-case” | | | | | |
| **Min** | 13.8 | 0.5 | 6.1 | 22.0 | |
| **Max** | 100.6 | 2.5 | 7.7 | 52.0 | |
| **Geomean** | 36.8 | 1.2 | 7.2 | 32.1 | |

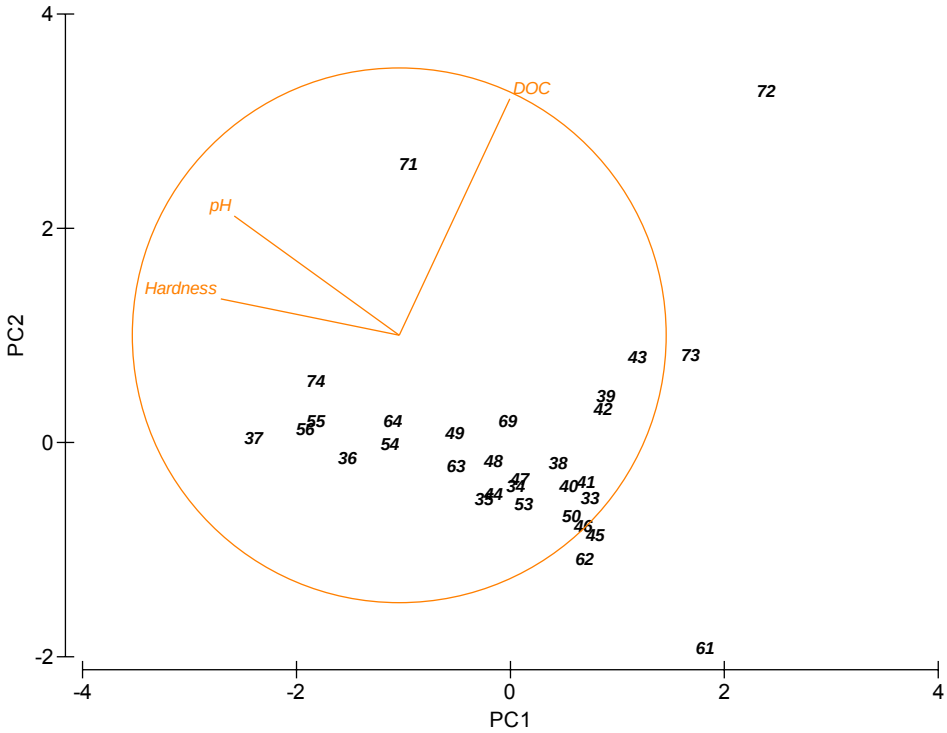


**Figure 1.** **Ordination of the results of a Principal Components Analysis (PCA) of *Ceriodaphnia dubia* test media physicochemistry**

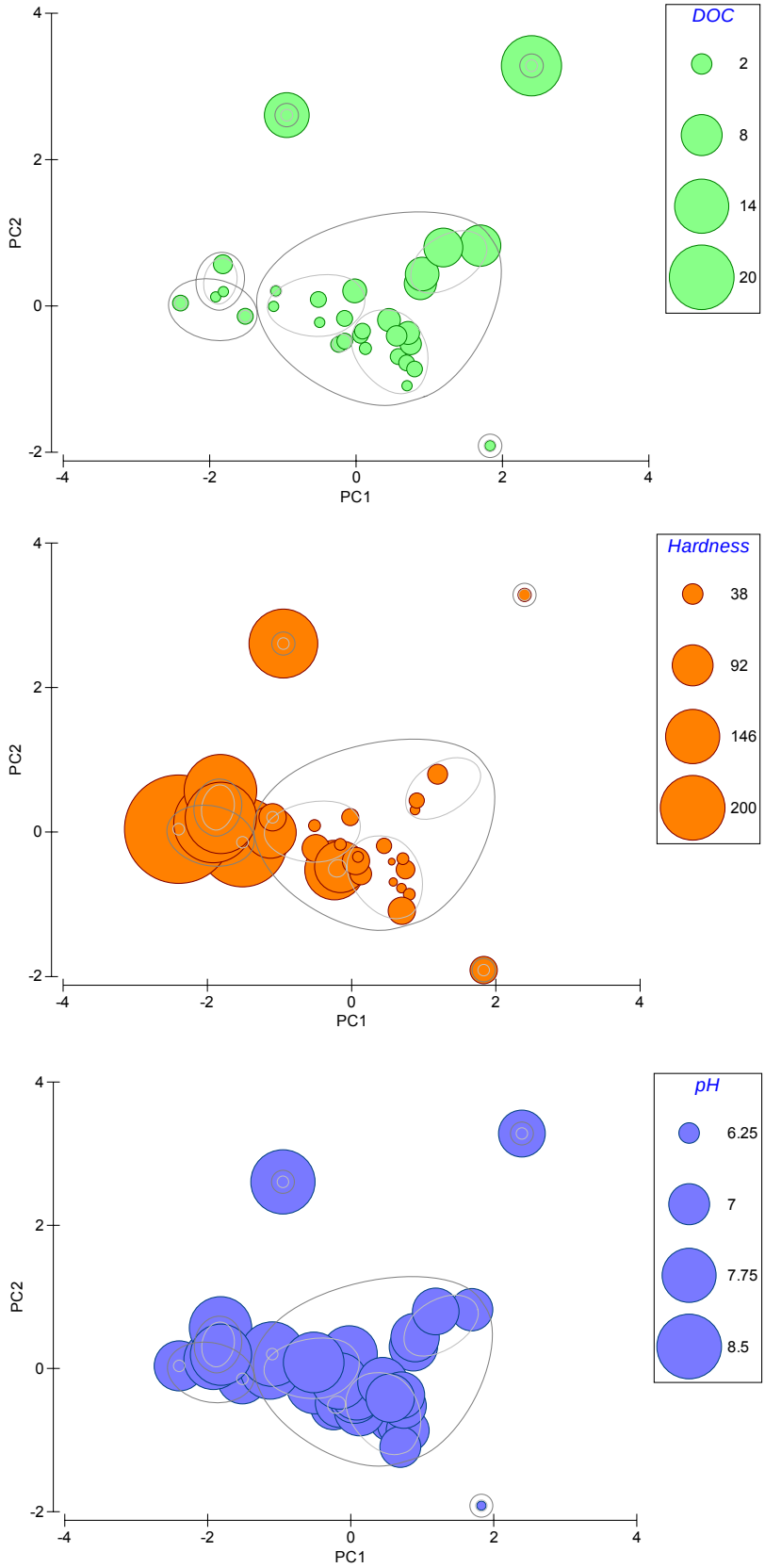


**Figure 2.** ***C. dubia*** **PCA ordination with values of DOC (mg C.L$^{-1}$), pH and hardness (mg.L$^{-1}$) shown as different sized bubbles. Test with high DOC are located in the top right of the plot, whist increasing pH and hardness move tests to the left. A cluster of tests with similar physicochemistry consistent with 'high bioavailability' are present in the bottom right of the ordination.**

### *Pimephales promelas* (fathead minnow)

Ten individual NOEC/$EC_{10}$ values for chronic tests conducted with *Ceriodaphnia dubia* were available for PNEC derivation (Table 2). The geometric mean NOEC/EC10 of all the available tests was 109.46 µg dissolved Pb. $L^{-1}$. PCA Eigenvalues 1 and 2 explain 54.2 and 26.6 % of the variability in the dataset, respectively (Figure 3). The variability in DOC and hardness is nearly completely explained by Principal Components 1 and 2. The influence of DOC, hardness and pH on the resulting clustering of tests can be interpreted from Figure 4. A cluster of tests characterised by low DOC, low hardness and circumneutral pH is apparent at the top right of the ordination. A geometric mean NOEC/$EC_{10}$ of the cluster of four "similar" tests consistent with "reasonable worst case" bioavailability is 29.29 µg dissolved Pb.$L^{-1}$.

**Table 2. Complete *P. promelas* dataset of chronic tests conducted under comparable methodology. "Reasonable worst-case" sub-set identified in bold.**

| CSR Test number | NOEC/$EC_{10}$ (µg.$L^{-1}$) | DOC (mg.$L^{-1}$ C) | pH | Hardness (mg.$L^{-1}$) | Reference |
|---|---|---|---|---|---|
| **151** | **20.0** | **1.2** | **7.4** | **19** | Grosell et al. 2006b |
| **152** | **39.6** | **1.2** | **7.2** | **47** | Grosell et al. 2006b |
| 153 | 41.8 | 1.2 | 7.2 | 104 | Grosell et al. 2006b |
| 154 | 175.5 | 2 | 8 | 22 | Grosell et al. 2006b |
| 155 | 357.4 | 2.6 | 7.9 | 21 | Grosell et al. 2006b |
| 156 | 448.9 | 7.3 | 8 | 25 | Grosell et al. 2006b |
| 157 | 1634.9 | 10.5 | 7.9 | 25 | Grosell et al. 2006b |
| **158** | **29.3** | **1.4** | **6.4** | **24.6** | Grosell, 2010d |
| **159** | **31.7** | **1.3** | **7.46** | **23.5** | Grosell, 2010d |
| 160 | 174.4 | 1.5 | 8.22 | 23.8 | Grosell, 2010d |
| Summary based on "all data" | | | | | |
| **Min** | 20.0 | 1.2 | 6.4 | 19.0 | |
| **Max** | 1634.9 | 10.5 | 8.2 | 104.0 | |
| **Geomean** | 109.46 | 2.13 | 7.55 | 28.62 | |
| Summary based on "Reasonable worst-case" | | | | | |
| **Min** | 20.0 | 1.2 | 6.4 | 19.0 | |
| **Max** | 39.6 | 1.4 | 7.5 | 47.0 | |
| **Geomean** | 29.29 | 1.27 | 7.10 | 26.80 | |

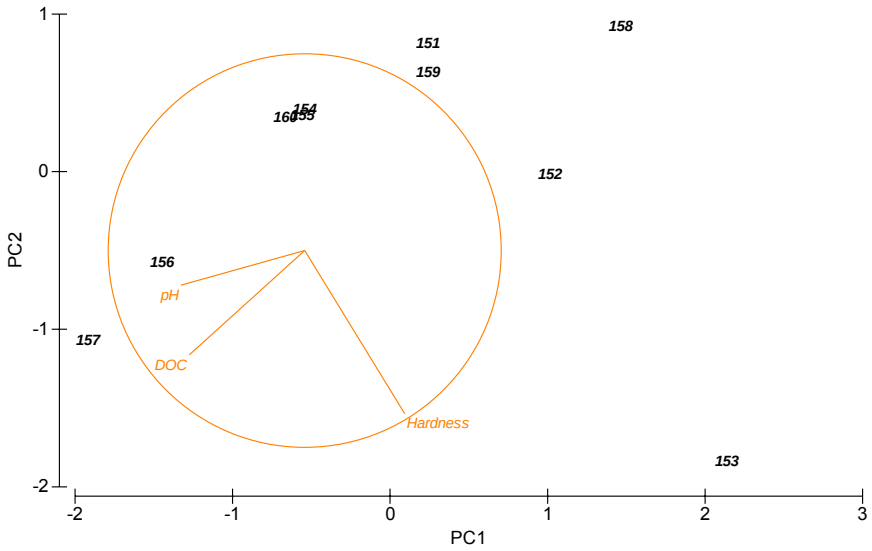


**Figure 3** **Ordination of the results of a Principal Components Analysis (PCA) of *Pimephales promelas* test media physicochemistry**

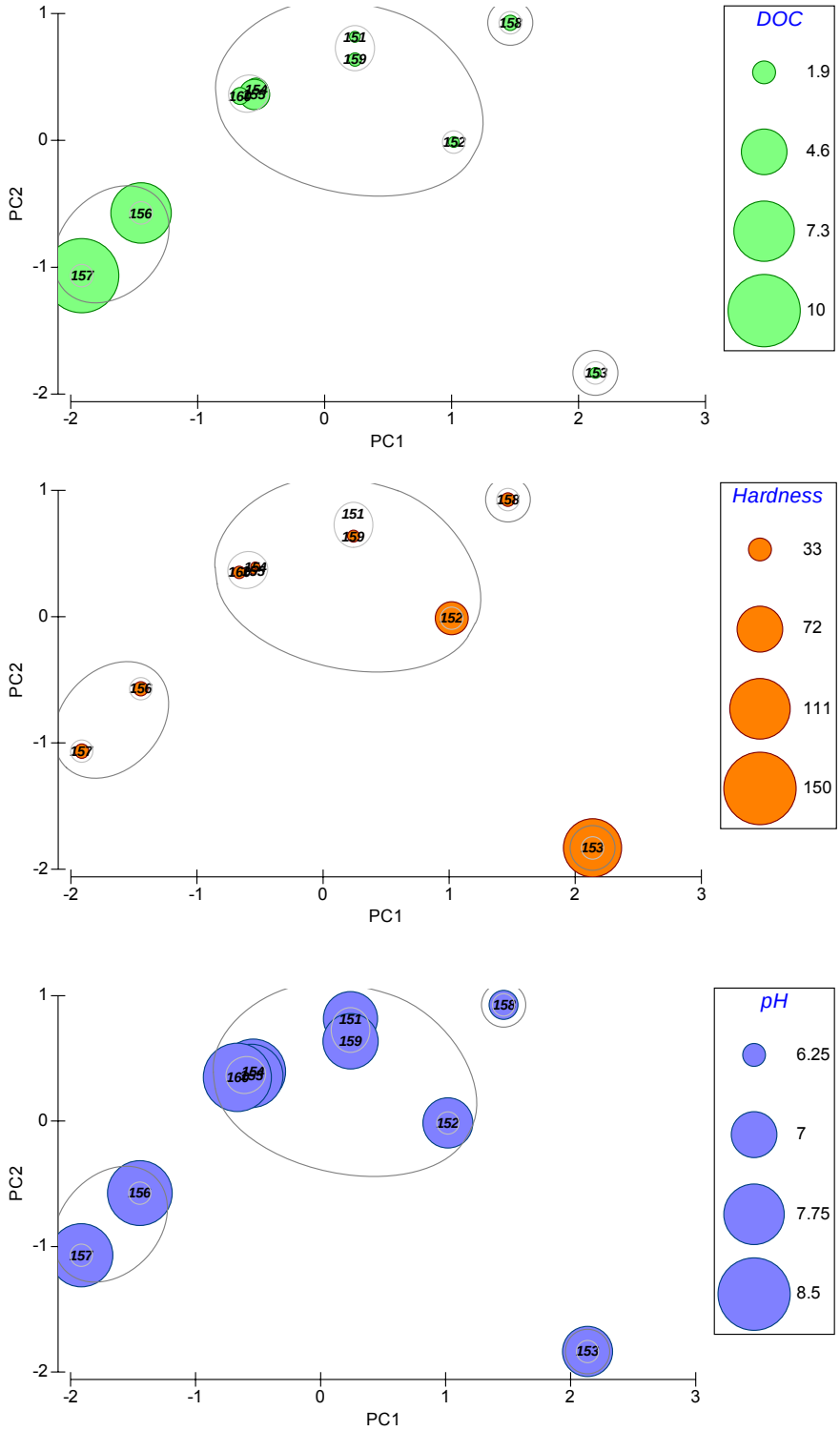


**Figure 4.** ***P. promelas*** **PCA ordination with values of DOC (mg C.L$^{-1}$), pH and hardness (mg.L$^{-1}$) shown as different sized bubbles. Test with high DOC are located in the bottom left of the plot, whilst increasing hardness moves tests to the bottom right. A cluster of four tests (151, 152, 158 and 159) with similar physicochemistry consistent with 'high bioavailability' are present in the top right of the ordination.**

## *Lemna minor* (Duckweed)

Seven individual NOEC/$EC_{10}$ values for chronic tests conducted with *Lemna minor* were available for PNEC derivation (Table 3). The geometric mean NOEC/EC10 of all the available tests was 572.79 µg dissolved Pb. $L^{-1}$. PCA Eigenvalues 1 and 2 explain 73.8 and 25.1 % of the variability in the dataset, respectively (Figure 5). The variability in DOC and hardness is nearly completely explained by Principal Components 1 and 2. The influence of DOC, hardness and pH on the resulting clustering of tests can be interpreted from Figure 6. A single test (106) is characterised by low DOC, low hardness and circumneutral pH. The NOEC/$EC_{10}$ from this test of 104.0 µg dissolved Pb.$L^{-1}$can be considered to be consistent with "reasonable worst case" bioavailability.

**Table 3. Complete *Lemna minor* dataset of chronic tests conducted under comparable methodology. "Reasonable worst-case" sub-set identified in bold.**

| CSR Test ID | NOEC/$EC_{10}$ (µg.$L^{-1}$) | DOC (mg.$L^{-1}$ C) | pH | Hardness (mg.$L^{-1}$) | Reference |
|---|---|---|---|---|---|
| **106** | **104.0** | **0.7** | **7.86** | **29** | Aquatox, 2010 |
| 107 | 973.4 | 6.9 | 7.17 | 56 | Aquatox, 2010 |
| 108 | 1109.5 | 4.9 | 8.32 | 266 | Aquatox, 2010 |
| 109 | 574.4 | 0.5 | 8.55 | 172 | Aquatox, 2010 |
| 110 | 647.6 | 1.4 | 8.35 | 77 | Aquatox, 2010 |
| 111 | 782.6 | 12.5 | 5.69 | 8 | Aquatox, 2010 |
| 112 | 618.7 | 3.6 | 8.06 | 33 | Aquatox, 2010 |
| Summary based on "all data" | | | | | |
| **Min** | 104.0 | 0.5 | 5.7 | 8.0 | |
| **Max** | 1109.5 | 12.5 | 8.6 | 266.0 | |
| **Geomean** | 572.79 | 2.57 | 7.65 | 54.94 | |
| Summary based on "Reasonable worst-case" | | | | | |
| **Min** | 104.0 | 0.7 | 7.86 | 29 | |
| **Max** | 104.0 | 0.7 | 7.86 | 29 | |
| **Geomean** | 104.0 | 0.7 | 7.86 | 29 | |

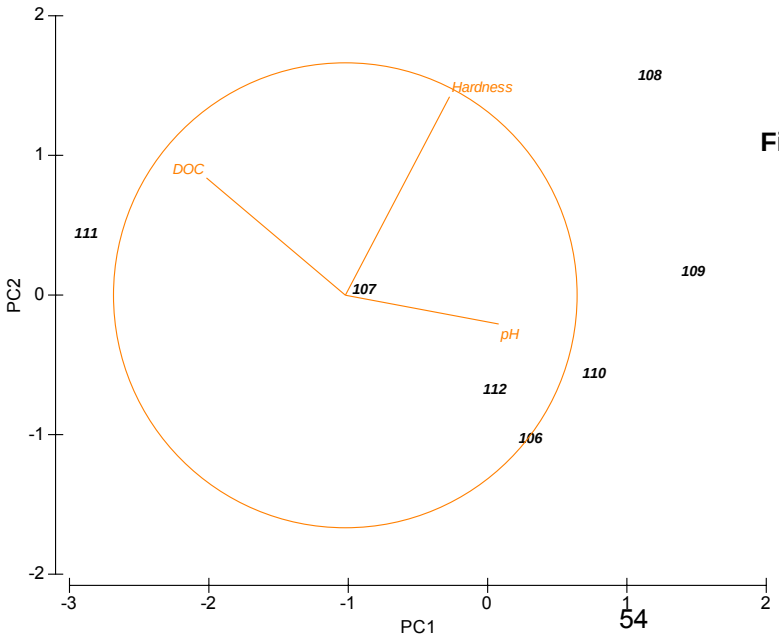


**Figure 5 Ordination of the results of a Principal Components Analysis (PCA) of *Lemna minor* test media physicochemistry**

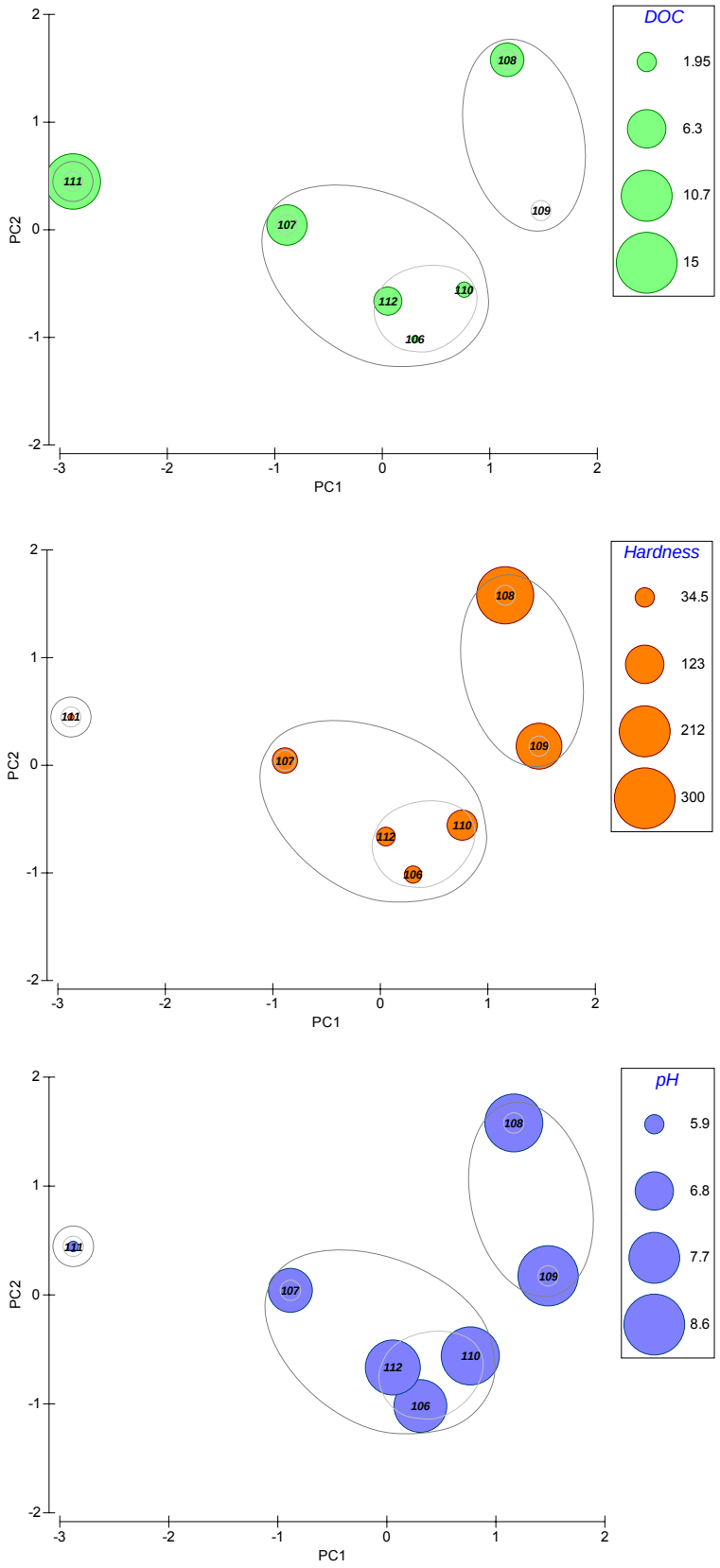


**Figure 6.** ***L. minor*** **PCA ordination with values of DOC (mg C.L$^{-1}$), pH and hardness (mg.L$^{-1}$) shown as different sized bubbles. Test with high DOC are located in the left of the plot, whilst increasing hardness moves tests to the top right. A single tests (106), located at the bottom left of the plot can be considered as consistent with 'high bioavailability'**

### *Pseudokirchneriella subcapitata* (Green algae)

Seven individual NOEC/$EC_{10}$ values for chronic tests conducted with *P. subcapitata* were available for PNEC derivation (Table 4). The geometric mean NOEC/EC10 of all the available tests was 15.25 µg dissolved Pb. $L^{-1}$. PCA Eigenvalues 1 and 2 explain 67.4 and 25.4 % of the variability in the dataset, respectively (Figure 5). The variability in DOC and pH is nearly completely explained by Principal Components 1 and 2. The influence of DOC, hardness and pH on the resulting clustering of tests can be interpreted from Figure 8. A cluster of tests characterised by low DOC, low hardness and circumneutral pH is apparent on the right hand side of the ordination. A geometric mean NOEC/$EC_{10}$ of the cluster of four "similar" tests consistent with "reasonable worst case" bioavailability is 8.42 µg dissolved Pb.$L^{-1}$.

**Table 4. Complete *Pseudokirchneriella subcapitata* dataset of chronic tests conducted under comparable methodology. "Reasonable worst-case" sub-set identified in bold.**

| CSR Test ID | NOEC/$EC_{10}$ (µg.$L^{-1}$) | DOC (mg.$L^{-1}$ C) | pH | Hardness (mg.$L^{-1}$) | Reference |
|---|---|---|---|---|---|
| **208** | **8.8** | **1.9** | **6.73** | **24** | De Schamphelaere & Janssen, 2010 |
| **209** | **5.4** | **1.8** | **7.28** | **24** | De Schamphelaere & Janssen, 2010 |
| **210** | **23.4** | **2.0** | **7.71** | **24** | De Schamphelaere & Janssen, 2010 |
| 212 | 93.0 | 17.4 | 7.34 | 174 | De Schamphelaere & Janssen, 2010 |
| 213 | 34.1 | 9.7 | 7.86 | 263 | De Schamphelaere & Janssen, 2010 |
| **216** | **4.5** | **1.9** | **7.28** | **24** | De Schamphelaere & Janssen, 2010 |
| 217 | 12.0 | 9.0 | 7.28 | 24 | De Schamphelaere & Janssen, 2010 |
| Summary based on "all data" | | | | | |
| **Min** | 4.5 | 1.8 | 6.7 | 24.2 | |
| **Max** | 93.0 | 17.4 | 7.9 | 262.7 | |
| **Geomean** | 15.25 | 4.10 | 7.35 | 45.10 | |
| Summary based on "Reasonable worst-case" | | | | | |
| **Min** | 4.5 | 1.8 | 6.7 | 24.2 | |
| **Max** | 23.4 | 2.0 | 7.7 | 24.2 | |
| **Geomean** | 8.42 | 1.89 | 7.24 | 24.19 | |

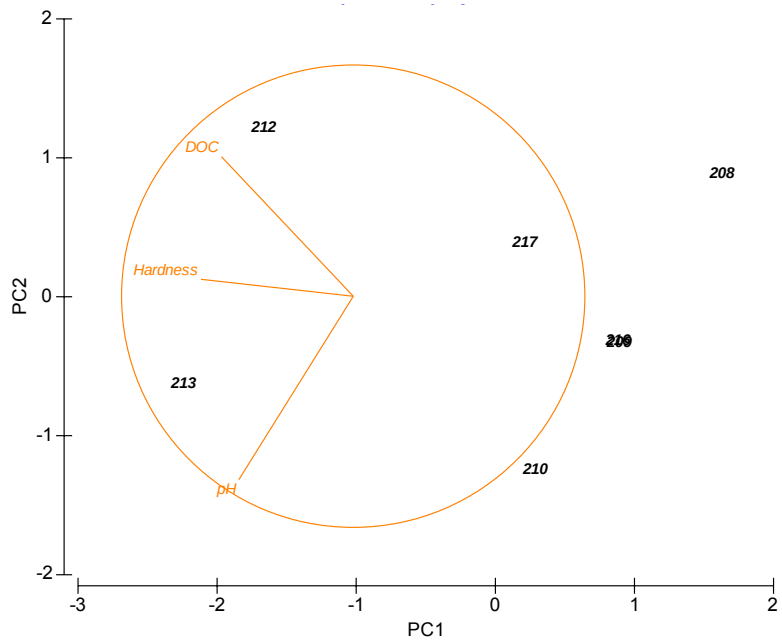


**Figure 7** **Ordination of the results of a Principal Components Analysis (PCA) of *P. subcapitata* test media physicochemistry**

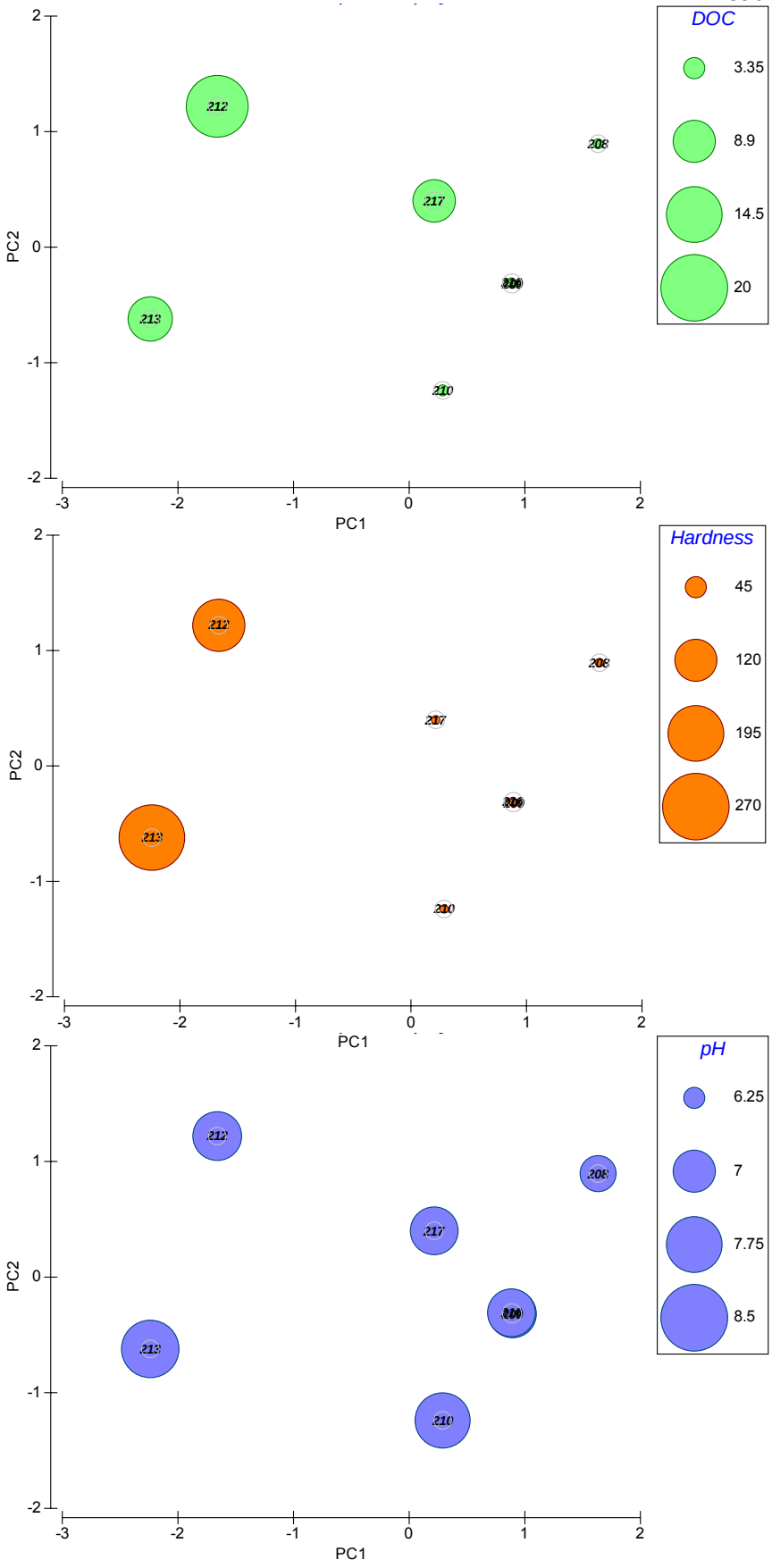


**Figure 8** ***P. subcapitata*** **PCA ordination with values of DOC (mg C.L$^{-1}$), pH and hardness (mg.L$^{-1}$) shown as different sized bubbles. Test with high DOC are located in the top left of the plot, whilst increasing hardness also moves tests to the left. A group of test with similar physicochemistry consistent with 'high bioavailability (208, 209, 210, 216) is located at the right hand side of the plot.**

### *Philodina rapida* (rotifer)

Five individual NOEC/$EC_{10}$ values for chronic tests conducted with *P. rapida* were available for PNEC derivation (Table 5). The geometric mean NOEC/EC10 of all the available tests is 9.89 µg dissolved Pb. $L^{-1}$. PCA Eigenvalues 1 and 2 explain 57.1 and 35.0 % of the variability in the dataset, respectively (Figure 9). The variability in hardness and pH is nearly completely explained by Principal Components 1 and 2. The influence of DOC, hardness and pH on the resulting clustering of tests can be interpreted from Figure 10. A cluster of tests characterised by low DOC, low to moderate hardness and circumneutral pH is apparent on the right hand side of the ordination. A geometric mean NOEC/$EC_{10}$ of two "similar" tests (113 and 114) consistent with "reasonable worst case" bioavailability is 10.66 µg dissolved Pb.$L^{-1}$.

**Table 5 Complete *P. rapida* dataset of chronic tests conducted under comparable methodology. "Reasonable worst-case" sub-set identified in bold.**

| CSR Test number | NOEC/$EC_{10}$ (µg.$L^{-1}$) | DOC (mg.$L^{-1}$ C) | pH | Hardness (mg.$L^{-1}$) | Reference |
|---|---|---|---|---|---|
| **113** | **10.5** | **0.9** | **8.2** | **87** | Grosell, 2010c |
| **114** | **10.8** | **1.1** | **7.3** | **133** | Grosell, 2010c |
| 115 | 2.4 | 3.4 | 7.2 | 5 | Grosell, 2010c |
| 116 | 12.3 | 8.1 | 7.3 | 12 | Grosell, 2010c |
| 117 | 28.2 | 16.9 | 8.4 | 26 | Grosell, 2010c |
| Summary based on "all data" | | | | | |
| **Min** | 2.4 | 0.9 | 7.2 | 5.0 | |
| **Max** | 28.2 | 16.9 | 8.4 | 133.0 | |
| **Geomean** | 9.89 | 3.41 | 7.66 | 28.27 | |
| Summary based on "Reasonable worst-case" | | | | | |
| **Min** | 10.5 | 0.9 | 7.3 | 87.0 | |
| **Max** | 10.8 | 1.1 | 8.2 | 133.0 | |
| **Geomean** | 10.66 | 0.99 | 7.74 | 107.57 | |

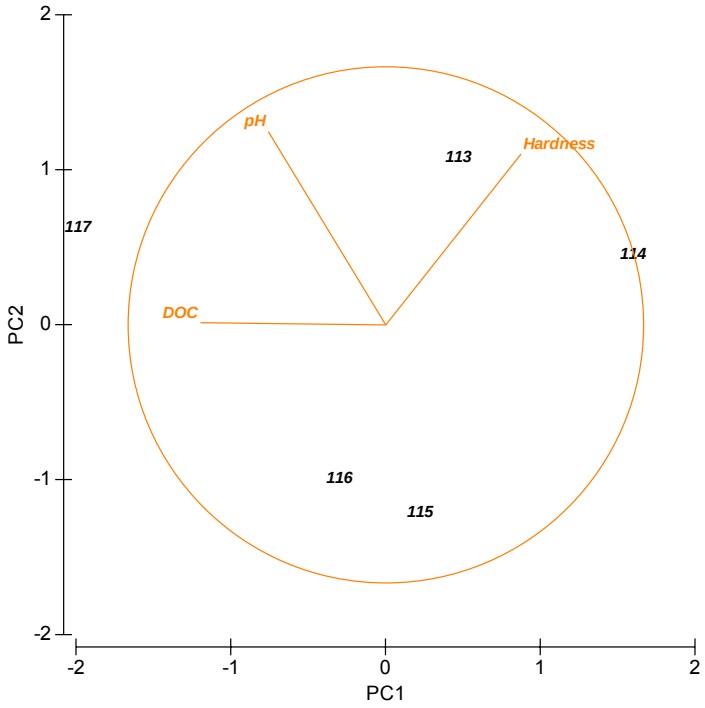


**Figure 9** **Ordination of the results of a Principal Components Analysis (PCA) of *P. rapida* test media physicochemistry**

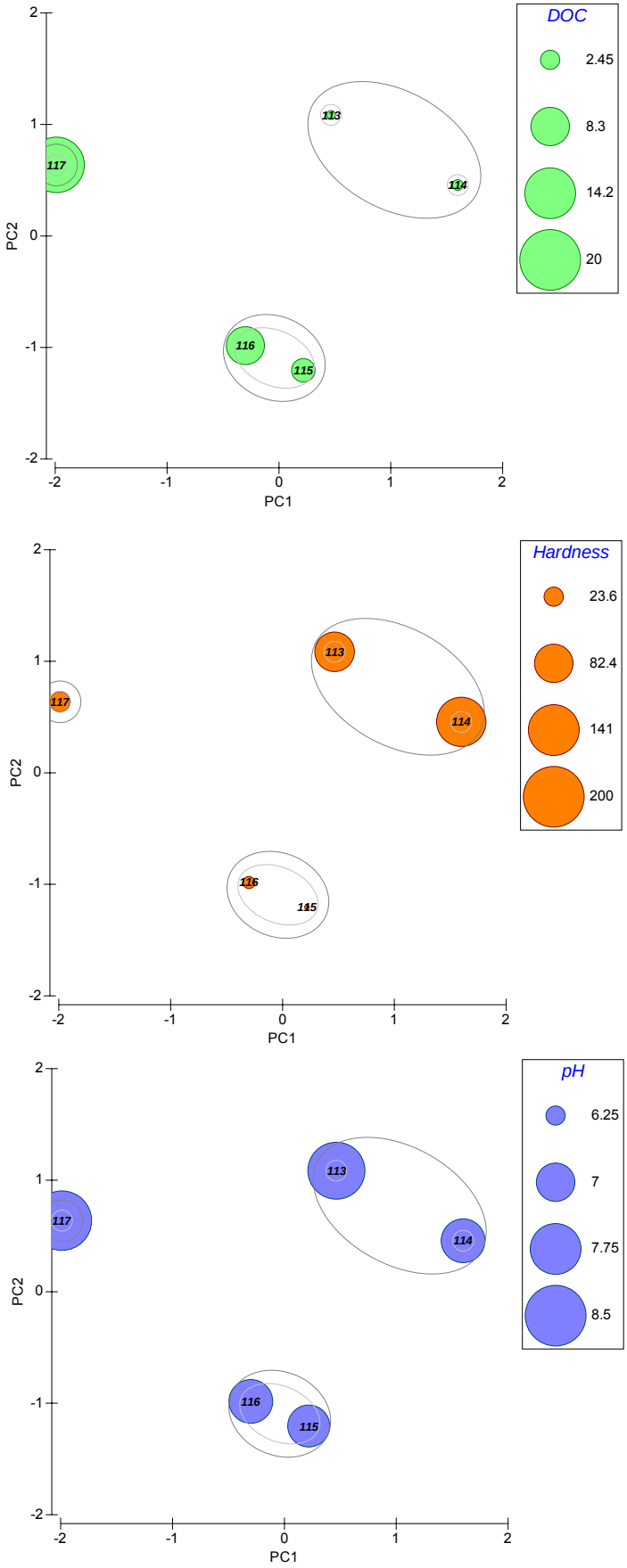


**Figure 10.** ***P. rapida*** **PCA ordination with values of DOC (mg C.L$^{-1}$), pH and hardness (mg.L$^{-1}$) shown as different sized bubbles. Test with high DOC are located in the left of the plot, whilst increasing hardness moves tests to the top right. A group of two tests with similar physicochemistry consistent with 'high bioavailability (113 and 114) is located at the top right hand side of the plot.**

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Parametrix 2009. Chronic toxicity of lead to the cladoceran, *Ceriodaphnia dubia*, under varying calcium and pH water quality conditions. Research Report. Parametrix Environmental Research Laboratory, Oregon, USA.

Parametric 2010. Chronic toxicity of lead to the fathead minnow, *Pimephales promelas*, in natural waters. Research Report. Parametrix Environmental Research Laboratory, Oregon, USA.

# ANNEX 3

## Application of the OECD Principles for the Validation of QSARs to the DOC correction for the Aquatic Toxicity of Pb

### Principle 1 (Defined Endpoint)

1.1 *A clear definition of the scientific purpose of the model*

The purpose of the model is to take account of the protective effect of DOC on chronic Pb toxicity to aquatic organisms

1.2 *The potential of the model to address a clearly defined regulatory need*

The model enables quality standards for Pb to be adjusted to take account of the effect of DOC on Pb toxicity, thus increasing the ecological relevance of the standard. The model predicts a threshold level for dissolved Pb which is protective of the effects of dissolved Pb on aquatic organisms.

1.3 *Important experimental conditions that affect the measurement and therefore the prediction*

Both pH and water hardness also affect the toxicity of Pb, but are not directly taken into account in the model. However, water hardness and pH were variable in the experimental datasets from which the regression slopes were calculated. This should mean that their influence on toxicity is partially accounted for. DOC is the dominant variable explaining toxicity.

1.4 *The units of measurement of the endpoint*

The endpoint is measured in units of $\mu g\ l^{-1}$ dissolved Pb

### Principle 2 (Defined Algorithm)

2 *An explicit definition of the equation, including definitions of all descriptors*

$$PNEC_{site} = PNEC_{reference} + (1.2 \times (DOC - DOC_{reference}))$$

| | |
|---|---|
| $PNEC_{site}$ | is the Predicted No Effect Concentration at the site under consideration |
| $PNEC_{reference}$ | EQS for a sensitive reference condition |
| DOC | Dissolved Organic Carbon at the site under consideration |
| $DOC_{reference}$ | average Dissolved Organic Carbon (DOC) concentration in the ecotoxicity tests that the $PNEC_{reference}$ is based upon, 1.0 mg $l^{-1}$ |

### Principle 3 (Defined Domain of Applicability)

3.1 *a description of any limits on the models applicability*

The model has been shown to provide protective estimates for validation data over the following range of water quality conditions:

DOC < 17 mg $l^{-1}$, pH between 6.0 and 8.5, hardness > 5 mg $l^{-1}$

3.2 *rules describing the modulatory effects of the local environment*

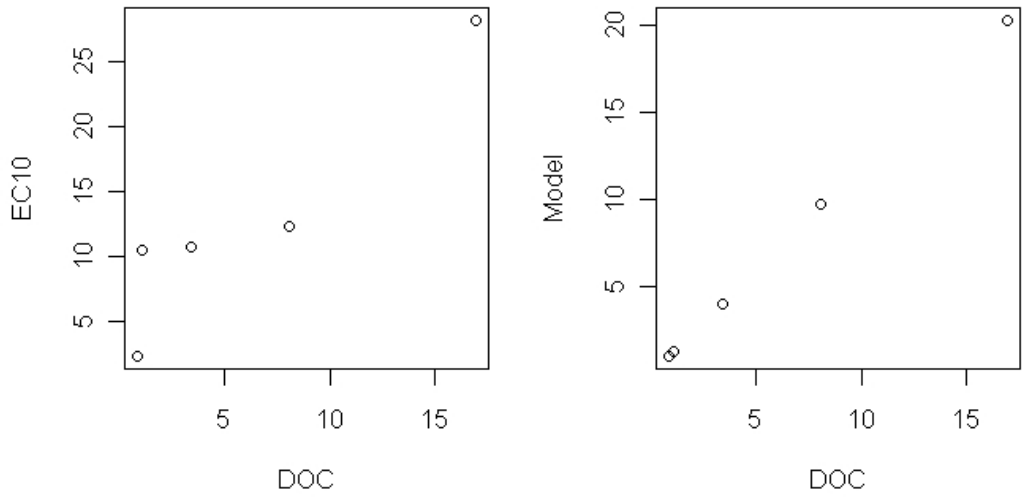
Low pH and high water hardness can provide protective effects against Pb toxicity, although the effects are less important overall than the effect of DOC and are not taken into account in the model. Increasing DOC concentrations result in an increase in the NOEC/EC10 for an organism. The model should only be applied when the water quality conditions (pH, DOC, and hardness) are within the range stated in 3.1.

3.3 *inclusion/exclusion rules that define the ranges for which the model is applicable*

See 3.1 for water quality conditions that the model can be applied to.

3.4 *expression of how the descriptor values of the chemicals in the training set are distributed in relation to the endpoint values predicted by the model*

The figure shows a quantile-quantile plot of the descriptor values (DOC) with the endpoint values (EC10) of the training dataset (left) and a quantile-quantile plot of the descriptor values (DOC) with the endpoint values (EC10) resulting from predictions (right).




**Principle 4A (Internal Performance)**

4.1 *Full details of the training set*

Data for the effect of Pb on *P. rapida*

4.2 *Raw or processed data used in development*

Calculated EC10 values for the effect of Pb on *P. rapida*

4.3 *Approach used to select descriptors*

Multivariate analysis of the influence of water chemistry on Pb NOEC/EC10 revealed that DOC alone was able to account for more variability in NOEC/EC10 values than other parameters.

4.4 *Specification of statistical methods applied*

Linear regression analysis between EC10 and DOC concentration for *P. rapida*

4.5 *Basic goodness of fit statistics*

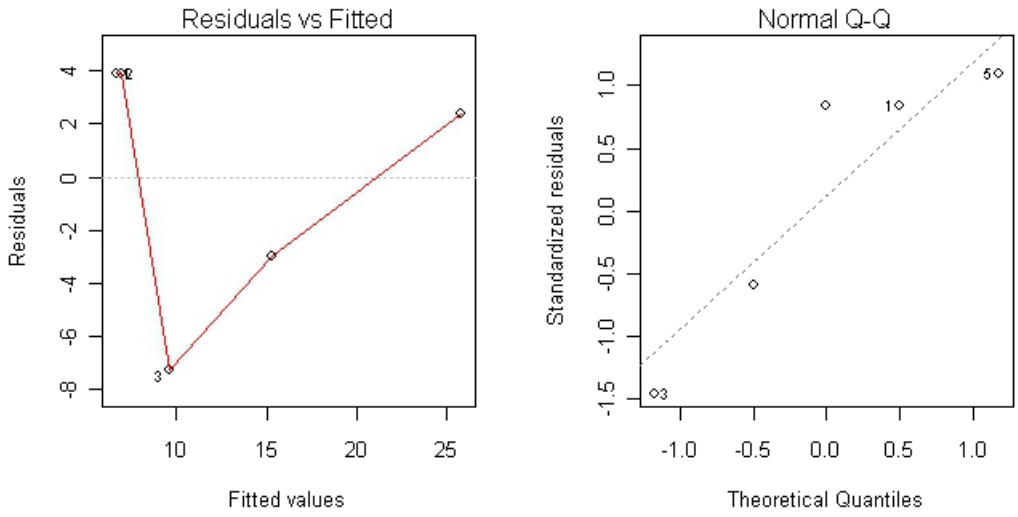
Adjusted R-squared: 0.634, p-value: 0.06696

4.6 *Was cross-validation or resampling performed*

No

4.7 *Internal performance of the model in relation to the quality of the training set*

As the model is intended to produce predictions which are protective of the results of the ecotoxicity tests the absolute ability of the model to predict either the results of the training or testing datasets is of limited importance. It is more important that the predictions are protective of the measured endpoints than that they are predicted accurately; indeed accurate predictions for all organisms would not ensure protection of sensitive organisms. The left hand figure below shows the residuals from the predictions vs. the fitted values, and indicates that there is some unexplained variation (this is likely to be due to variation in pH and hardness). The top right figure shows that the residuals are approximately normally distributed. The small size of the training dataset precludes a detailed analysis




**Principle 4B (Predictivity)**

4.8 *Has the model been validated by using a test set that is independent of the training set*

Yes

4.9 *Details of external validation set*

137 ecotoxicity test results with measured DOC concentrations, covering 19 species

4.10a *Approach used to select the test data*

Measured, or estimated, DOC concentrations available for the test

4.10b *Was the external set sufficiently large and representative of the training data set*

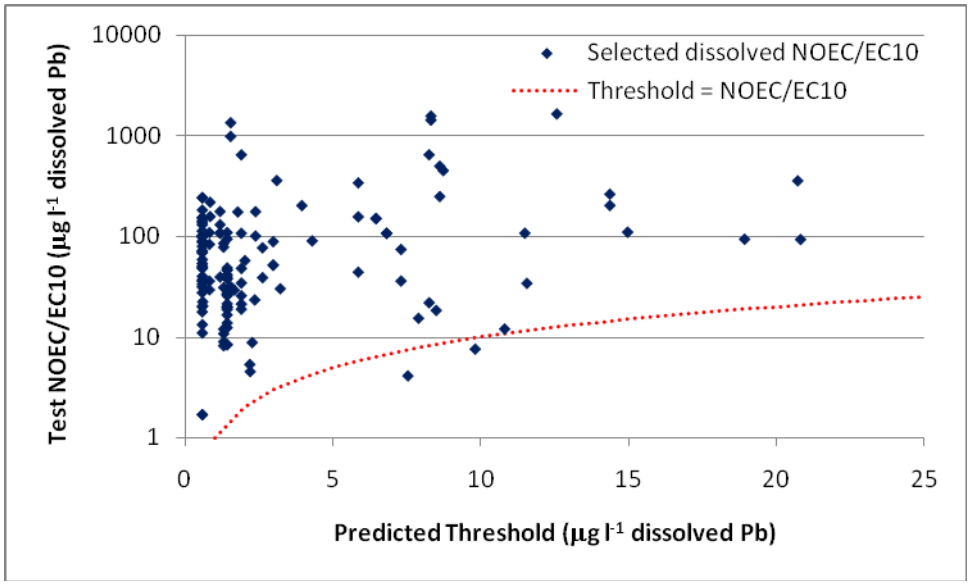
Ecotoxicity data for 19 different species, covering water quality conditions between pH 5.6 and 8.6, DOC concentrations between 0.5 and 17.4 mg $l^{-1}$, and Ca concentrations between 2 and 142 mg $l^{-1}$.

4.10c *Specification of the statistical method used to assess the predictive performance*

Is the predicted $PNEC_{site}$ lower than the NOEC/EC10 for the test?

4.10d *Statistical analysis of the predictive performance of the model*

Of 137 test results which the model was compared against only two cases (1.5%) resulted in predictions which were not protective of the observed test data. One of these was a test with *C. dubia* which was considered to be an outlier from the other available test data for this species as the other 55 test results for this species were all protected. The other was *L. stagnalis* and was also considered to be an outlier as the other five test results were all protected by the predictions. Neither of the two tests which were not protected were conducted under extreme conditions of pH, DOC, hardness, or Ca which might have explained increased sensitivity. The figure shows a comparison between the predicted threshold using the model and the measured NOEC/EC10 values for 137 experiments included in the testing dataset. Both of the two test results which are not predicted conservatively by the model are within a factor of 2 of the predicted threshold from the model.




4.10e *Evaluation of the predictive performance of the model*

The model predicted a $PNEC_{site}$ value which was lower than the observed NOEC/EC10 values in 98.5% of the 137 available ecotoxicity tests on organisms other than the organism from which the model was developed.

4.10f *Comparison of the predictive performance of the model against previously-defined quantitative performance criteria*

Models of metals bioavailability which have already been accepted for regulatory use have typically been required to provide predictions of toxicity to a specific organism to within a factor of two of the true result. The two test values from the validation set which are not protected by the model are both within a factor of two of the predicted threshold. The predicted thresholds are conservative by over a factor of two for all tests from the validation set with the exception of a further two tests, and protective by over a factor of three for all except a further seven tests. The thresholds predicted by the model are therefore protective by more than a factor of three for 92.0% of the validation dataset.

**Principle 5 (Mechanistic Interpretation)**

5.1 *Properties of molecules containing the substructure*

Not relevant to this model

5.2 *Interpretation that is consistent with a known mechanism of biological action*

There is considerable evidence that DOC reduces the chemical availability of Pb in chronic ecotoxicity tests, but currently insufficient evidence to propose a biotic ligand based model that includes other physicochemical variables. Evidence from the validation dataset indicates that a protective effect is seen for all species for which tests have been performed a range of DOC concentrations.

5.3 *Literature references that support the mechanistic basis*

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Grosell M, Gerdes R, Brix K. 2006 CBP Part C. 143:473-483.

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5.4 *Was the mechanistic basis of the model determined a priori or a posteriori*

The mechanistic basis for the model was selected *a posteriori*