Data compilation, selection and derivation of PNEC values for the aquatic compartment

Zinc example

1. Regulatory context

Zinc metal (Zn) and five zinc compounds, i.e. zinc oxide (ZnO), zinc chloride ($ZnCl_2$), zinc sulphate ($ZnSO_4$), zinc phosphate ($Zn_3(PO4)_2$ and zinc distearate ($(C_{18}H_{35}O_2)_2Zn$) were prioritized under EU Regulation EEC/793/93 in September 1995. This implied that a full risk assessment (RA) for Zn needed to be carried out, following the guidelines detailed in the Technical Guidance Document (TGD) on Risk Assessment for New and Existing Substances (EU, 2003). The Netherlands acted as the Rapporteur country in this process, in close collaboration with the international zinc industry. The draft final European Union Risk Assessment Report (EU RAR) on zinc (environmental part) has become available in 2006 (EU, 2006) after thorough review by the Technical Committee on New and Existing Substances, which was comprised of technical representatives from the EU Member States. A final peer review was provided by the Scientific Committee on Health and Environmental Risks (SCHER) in November 2007 (Http://ec.europa.eu/health/ph_risk/committees/04_scher/docs/scher_o_069.pdf1.3). The RA was published in 2008 (ECB, 2008). It is noted that both the effects data set and the bioavailability correction were updated under the REACH registration process. This new evidence is not included in this assessment.

2. General process

Environmental risks are typically characterized in the risk assessment framework by considering the ratio between exposure concentrations and critical effect concentrations. In OECD countries, critical effect concentrations are based on Predicted No Effect Concentrations (PNEC), which are typically derived from long-term laboratory-based ecotoxicity tests using well-defined protocols on a limited number of species. Such information is usually retrieved from relevant literature and/or internationally recognized databases. Because the quality of the extracted data may vary considerably among individual source documents, it is important to evaluate all ecotoxicity data with regard to their adequacy for PNEC derivation and risk assessment. This document provides an example on how such evaluation for the freshwater aquatic compartment including criteria for acceptance (or rejection) of a study, was conducted for Zinc in the EU RA made by The Netherlands.

The following steps need to be accomplished in order to derive the critical effect concentrations (PNEC) of Zn for the freshwater compartment (Figure 1):

Figure 1: Stepwise approach used for the derivation of the freshwater PNEC value.

3. Example: Zinc Risk Assessment (ECB 2008)

3.1 DATA COMPILATION

The data on the toxicity of Zn to freshwater organisms were compiled from 3 main sources: open literature, internationally recognized databases, and industry sponsored research programs. A large dataset on the chronic ecotoxicity of Zn to freshwater organisms was compiled. All gathered data were further screened using the criteria as outlined in Section 3.2.

3.2 DATA QUALITY SCREENING

Each individual ecotoxicity data point was screened for quality before incorporation in the zinc ecotoxicity database based on the following criteria:

- data were retained for the following groups of organisms: *algae, invertebrates and fish*;
- data covered the following relevant endpoints: *survival, growth, hatching and/or reproduction*;
- the *pH, hardness and dissolved organic carbon (DOC) of the exposure media should be reported.* In cases where one or more of these variables were not reported in the original publication, the values of the missing variables were estimated based on, e.g. (i) monitoring data (e.g., in cases where test media were natural waters), (ii) published test guidelines (e.g., in cases where only a reference to a standard medium published in a standard testing guideline was given), (iii) charge balance and ionic strength considerations, etc.
- the data were from studies conducted according to approved *international standard test guidelines*. However data from *non-standardized tests* were also assessed;
- only *long-term or chronic toxicity data*, involving endpoints that are realized over periods of several days to years depending on the organism, were used;
- the tests were performed according to standard operational procedures, with a *detailed description of the methods* employed during toxicity testing;

- both nominal and *measured zinc concentrations* in the test concentrations are retained[1];
- a *clear concentration-response* was observed;
- the toxicity tests were performed with *soluble zinc salts (e.g., $ZnCl_2$, $ZnSO_4$). Data derived from the direct testing of the poorly soluble compounds or the metals were rejected*;
- the toxicity test results reflected *dissolved zinc concentrations* and were expressed as *µg Zn/L*;
- ecotoxicity threshold values, *L(E)C_{10}* or *NOEC values*, were derived using *proper statistical methods*.

3.3. DATA RELEVANCY SCREENING

Ecotoxicity data, especially those reported in the open literature, can be obtained under widely varying test conditions. Testing medium factors that were identified to significantly influence ecotoxicity data for zinc were a) background concentration in test medium and culture medium prior to testing: related to possible conditioning of organisms, see item "adaptation to natural background") and b) physico-chemical conditions influencing bioavailability (see corresponding item). To ensure that reported ecotoxicity data were relevant for the European environment, a number of data relevancy criteria were applied to the zinc dataset, in addition to the criteria for quality screening (3.2.):

- the range of the *physico-chemistry of the test media* (pH, hardness) were within the range of observed values in the EU, i.e. pH should be between 6 and 9, hardness between 24 and 250 mg/L $CaCO_3$;
- adaptation of the organisms to very low or very high zinc concentrations may influence the sensitivity to zinc. In that respect, if the dissolved *zinc concentration in the culture media* of the test organism was *below 1 µg/L*, the study was not retained. However, some studies conducted in natural waters at levels below 1 µg/l were maintained;

Only identified ecotoxicity data fulfilling the criteria mentioned under 3.2. and 3.3. were used for the freshwater PNEC derivation.

3.4. DATABASE DEVELOPMENT

Applying the above mentioned quality and relevancy screening criteria to the identified ecotoxicity data resulted in the selection of an extensive high quality database on the chronic ecotoxicity of zinc to freshwater organisms. Indeed, the database comprised 19 different 'species means' from 131 individual high quality L(E)C_{10}/NOEC values (35 individual L(E)C_{10}/NOEC values for algae; 55 for invertebrates; 41 for fish), covering good distribution of trophic levels and species families.

3.5. INCORPORATION OF BIOAVAILABILITY (DATA NORMALIZATION)

There is extensive evidence demonstrating the importance of bioavailability altering water quality factors on the toxicity of zinc towards aquatic organisms. Indeed, site-specific geochemical conditions (e.g. pH, Hardness, Dissolved Organic Carbon) influence the degree to which organisms take up zinc, and exhibit effects from such uptake. From a risk assessment perspective, it is therefore critical to consider bioavailability, as geographically distinct eco-regions, watersheds and sites will

[1] Nominal concentrations were checked to be corresponding to real test levels.

often show distinctive geochemical characteristics therefore leading to different critical effects concentrations (PNECs).

In the EU zinc RA, bioavailability was taken into account at the exposure side of the risk equation[2]. The effects database was not normalised, but a generic PNEC was derived (which was proven to correspond to realistic worst case water physico-chemistry).

For reasons of completeness and illustration, the zinc effects data are normalized in this example towards the following physico-chemical conditions using a state-of-the-art bioavailability model (BLM; BIOMET 2011): pH: 7.9, Ca: 40.1 mg/L, DOC: 2.1 mg/L.

3.6. DATA AGGREGATION

Normalized high quality ecotoxicity data are grouped/aggregated in order to avoid over representation of ecotoxicological data from one particular species. The following major rules were used to aggregate data:

- If several chronic NOEC/L(E)C_{10} values based on the same toxicological endpoint, were available for a given species, the values were averaged by calculating the geometric mean, resulting in the "species mean" NOEC/L(E)C_{10}.
- If several (geometric mean) chronic NOEC/L(E)C_{10} values based on different toxicological endpoints were available for a given species, the lowest (geometric value) value was selected.

After the data aggregation step, only one ecotoxicity value (i.e. the geometric mean for the most sensitive endpoint) was assigned to a particular species.

An overview of the normalized species mean NOEC/L(E)C_{10} value for the most sensitive endpoint is provided in Table 1.

Table 1: Selected freshwater normalized species mean ecotoxicity data to Zn for the most sensitive endpoint

Taxonomic group	Species	Most sensitive endpoint	Species mean NOEC/L(E)C_{10} value (µg/L)
Algae	*Pseudokirchneriella subcapitata*	Growth rate	12.6
	Chlorella sp.	Growth rate	28.3
Rotifer	*Anuraeopsis fissa*	Population growth	116.2
	Brachionus rubens	Population growth	116.2
Molluscs	*Dreissena polymorpha*	Mortality	179.2
	Potamopyrgus jenkinsi	Growth	39.1
Cladocerans	*Ceriodaphnia dubia*	Reproduction	34.4
	Daphnia magna	Reproduction	151.7
	Daphnia longispina	Reproduction	205.4
Insects	*Ephoron virgo*	Mortality	438.2
Amphipods	*Hyalella azteca*	Mortality, reproduction	43.3
Fish	*Jordanella floridae*	Growth	74.9

[2] Different as for other EU metal risk assessments (Cu, Ni, …) where ecotoxicity data sets and PNECs were normalized for bioavailability.

Taxonomic group	Species	Most sensitive endpoint	Species mean NOEC/L(E)C_{10} value (µg/L)
	Phoxinus phoxinus	Mortality, growth	110.8
	Pimephales promelas	Reproduction	214.2
	Oncorhynchus mykiss	Mortality	291.9
	Salvelinus fontinalis	Hatching	1476.5
	Danio rerio	Hatching	1218.3
	Salmo trutta	Hatching	182.4
	Cottus bairdi	Mortality	137.2

3.7 CALCULATION OF HC5 USING STATISTICAL EXTRAPOLATION METHODS

3.7.1 Estimation of the HC_5 from the species sensitivity distribution (SSD)

When a large data set for different taxonomic groups is available, the PNEC can be calculated using a HC5 value following from a statistical extrapolation method.

Usually, the log-normal distribution (e.g. the methods of Wagner & Løkke (1991) and Aldenberg & Jaworska (2000)) and the log-logistic distribution (Aldenberg & Slob, 1993) are pragmatic choices because of their mathematical properties (methods exist that allow for most in-depth analysis of various uncertainties). However, several other SSD curve fitting functions could also used in order to derive SSDs. The probability distribution fitted to the datasets used for the calculations of the 5th percentile values have been checked with the Anderson-Darling (A/D) goodness-of-fit test for normality and with the Kolmogorov-Smirnov (K/S) test[3]. In the zinc RA, the log-normal distribution was used, since it was proven statistically significant.

Fitting the distributions to the normalized toxicity data as mentioned in Table 1 revealed that the logistic distribution on the log-transformed toxicity data was the best fitting distribution. It must be emphasized that both distributions, i.e. the logistic and the normal distributions, were accepted at the significance level of 0.05 according to both the A/D and K/S goodness-of-fit statistics. An overview of the best fitting (i.e. logistic SSD on the log-transformed toxicity data) and normal SSD on the log-transformed toxicity data is presented in Figure 2 and 3.

[3] The Anderson-Darling goodness-of-fit test highlights differences between the tail of the distribution and the input data, while the Kolmogorov-Smirnov test focuses on differences in the middle of the distribution and is not very sensitive to discrepancies of fit in the tail of the distribution.

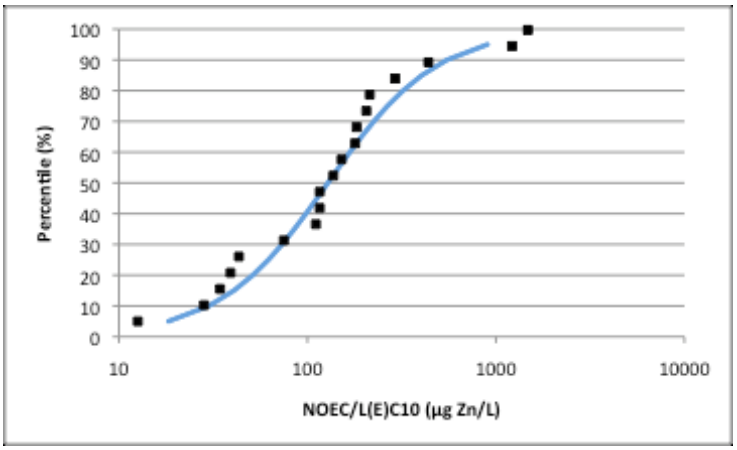

Figure 2: The cumulative frequency distributions of the normalized species mean NOEC/L(E)C_{10} values from the chronic Zn toxicity tests in the dataset of freshwater organisms. Geochemical parameters for this scenario were: pH = 7.9, Ca = 40.1 mg/L, DOC = 2.1 mg/L. Observed data and logistic distribution curve (best fitting curve) on the log-transformed toxicity data.

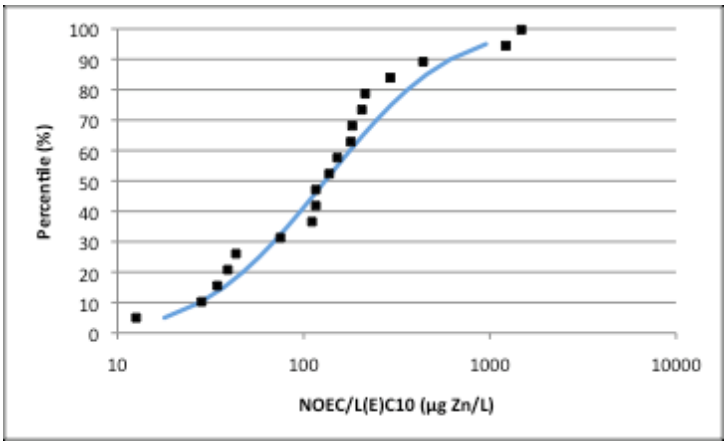

Figure 3: The cumulative frequency distributions of the normalized species mean NOEC/L(E)C_{10} values from the chronic Zn toxicity tests in the dataset of freshwater organisms. Geochemical parameters for this scenario were: pH = 7.9, Ca = 40.1 mg/L, DOC = 2.1 mg/L. Observed data and normal distribution curve on the log-transformed toxicity data.

From the SSD, a 5th percentile value (at the median confidence interval) is further calculated (i.e. median HC_5) using the software program ETx for the normal distribution (Van Vlaardingen et al., 2004) and using
the Aldenberg and Slob (1993) equation for the logistic SSD. The calculated median 5th percentile values (i.e. $HC_{5\text{-}50}$ value) for both distributions (with lower confidence limit) are shown in Table 2.

Table 2: Overview of the $HC_{5\text{-}50}$ value (µg/L) with confidence interval

Distribution function	**$HC_{5\text{-}50}$ value (µg/L)**	**Lower 95th % confidence limit (µg/L)**
Logistic (best fitting)	17.1	6.2
Normal	17.3	7.0

3.7. Selection of an appropriate assessment factor (AF) and derivation of the PNEC

To account for uncertainty, an assessment factor (AF) may be applied to the median HC_5. In general, such AFs vary between 1 and 5, and are determined on a case-by-case basis. The freshwater PNEC would therefore be calculated as follows:

Freshwater PNEC = median HC_5/ AF

Based on the available chronic NOEC/L(E)C_{10} data, the following points are considered when determining the AF:

- The overall quality of the database and the end-points covered (e.g. are all the compiled data representative of "true" chronic exposure ?),
- The diversity of the taxonomic groups covered by the database (e.g., do the databases contain at a minimum organisms belonging to the following 8 taxonomic groups ?, as defined by the London workshop (2001); Table 3),

Table 3: Taxonomic group requirements according to the criteria developed at the London workshop (2001)

	Taxonomic groups
1	Fish (usually tested species like salmons, bluegill, channel catfish, etc.)
2	A second family in the phylum Chordata (fish, amphibian, etc.)
3	A crustacean (e.g. cladoceran, copepod, ostracod, isopod, amphipod, crayfish etc.)
4	An insect (e.g. mayfly, dragonfly, damselfly, stonefly, caddisfly, mosquito, midge, etc.)
5	A family in a phylum other than Arthropoda or Chordata (e.g. Rotifera, Annelida, Mollusca, etc.)
6	A family in any order of insect or any phylum not already represented
7	Algae
8	Higher plants

- The number of species (e.g., does the SSD cover at least 10 different L(E)C_{10}/NOECs, and preferably more than 15 ?),
- Use of bioavailability models and approach for bioavailability correction (e.g., do the bioavailability models allow the toxicity data for all species to be normalized?),
- Statistical extrapolation (e.g., how well does the SSD fit the toxicity data?)
- Comparisons between field and mesocosm studies and the PNEC (e.g., is the PNEC value protective for the effects observed in mesocosm/field studies?).It is clear that the definition of the AF is function of the information available.

4. REFERENCES

Aldenberg T. & Jaworska J. S., 2000. Estimation of the hazardous concentration and fraction affected for normally distributed species sensitivity distributions. Ecotoxicology and Environmental Safety, 46, 1-18.

Aldenberg, T, & Slob, W. 1993. Confidence Limits for Hazardous Concentrations Based on Logistically Distributed NOEC Toxicity Data. Ecotoxicology and Environmental Safety 25(1), 48-63.

European Commission – Joint Research Centre, Institute for Health and Consumer Protection, European Chemicals Bureau (ECB). 2008. European Union Risk Assessment Report Zinc metal, Volume 42. Final report. (S.J. Munn et al. eds.) 812 pp.

London, workshop, 2001. Report of the expert consultation workshop on statistical extrapolation techniques for environmental effects assessments. Londen, VK, European Chemicals Bureau, 17-18 januari 2001.

Van Vlaardingen, P. L., T. P. Traas, A. M. Wintersen and T. Aldenberg. 2004. ETX 2.0. A program to calculate risk limits and fraction affected, based on normal species sensitivity distributions. Report no. 601501028/2004, National Institute for Public Health and the Environment (RIVM), Bilthoven, the Netherlands.

Wagner, C. & Lokke, H. 1991. Estimation of ecotoxicological protection levels from NOEC toxicity data. Water Research 25, 1237–1242.