SUPPORTING INFORMATION

Refinement and cross-validation of nickel bioavailability in PNEC-pro, a regulatory tool for site-specific risk assessment of metals in surface water.

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1. Detailed Analysis of Toxicity Data

Alona affinis

	n	EC10 or NOEC	Geomean EC	Source	Reference
Ni-RAR	1	25	25	Grey	(Deleebeeck et al., 2006)
PNEC-pro 5	2	25-25	25	Peer reviewed	Deleebeeck et al. 2007b
PNEC-pro 6	1	25	25	Peer reviewed	Deleebeeck et al. 2007b

The origin of the value of 25 µg/L in the Ni-RAR could not be traced. In the peer-reviewed paper of Deleebeeck, that probably describes the same experiment, this value is not mentioned. Since the Ni-RAR mentioned this value for hard water, the value is also included in PNEC-pro. The input parameters required for BLM calculations are retrieved from the peer reviewed study. PNEC-pro also assigned the NOEC of 25 µg/L to medium hard water. This value is removed from PNEC-pro 6 toxicity database, in order to harmonize with the RAR.

Ankistrodesmus falcatus

	n	EC10 or NOEC	Geomean EC	Source	Reference
Ni-RAR	2	13.6-59.4	28.4	Grey	(Deleebeeck *et al.*, 2006)
PNEC-pro 5	2	24.6-43.0	32.5	Peer reviewed	(Deleebeeck et al., 2009a)
PNEC-pro 6	2	18.3-43.6	28.4	Peer reviewed	(Deleebeeck *et al.*, 2009a)

The EC10 values mentioned in the Ni-RAR originate from grey literature (Deleebeeck, 2006), and could not be verified. The same tests were probably published in a peer- reviewed paper of the same author in 2009. Although the water types are identical in the report and the peer reviewed paper, the reported EC10 values are not identical. In PNEC-pro 5 the NOEC values were used. For the PNEC-pro 6 we selected the EC10, if both were NOEC and EC10 were computed for the same test.

Brachionus calyciflorus

	n	EC10 or NOEC	Geomean EC	Source	Reference
Ni-RAR	6	103.9-1379.3	633	Grey	(Stubblefield et al., 2007)
PNEC-pro 5	No data				
PNEC-pro 6	5	4.7-1379.3	211	Peer reviewed	(Schlekat et al., 2010)

The Ni-RAR used a study that was not readily available on internet. The Ni-RAR contained insufficient information about the input parameters required for full BLM calculations. In 2010, a peer reviewed study was published which contained a full set of input parameters for BLM calculations. Therefore, these data are adopted in PNEC-pro 6.

Bufo terrestris

	n	EC10 or NOEC	Geomean EC	Source	Reference
Ni-RAR	5	640-900	640	Peer reviewed	(Fort et al., 2006)
PNEC-pro 5	5	640-900	640		
PNEC-pro 6	5	880-1430	900		

PNEC-pro 5 preferred NOECs over EC10, as did the Ni-RAR. PNEC-pro 6 used EC10 values.

Ceriodaphnia dubia

	n	EC10 or NOEC	Geomean EC	Source	Reference
Ni-RAR	Mortality (4) Reproduction (11)	5.3-15.3 2.8-44.2	9.2 6.9	Peer reviewed Grey	(Keithly et al., 2004, Wirtz et al., 2004, De Schamphelaere et al., 2006)
PNEC-pro 5 and 6	Mortality (4) Reproduction (6)	5.3-15.3 2.8-7.6	9.2 4.7	Peer reviewed Grey	(Keithly *et al.*, 2004, Wirtz *et al.*, 2004)

RAR and PNEC-pro databases both contain 6 data from Keithly et al. (2004) and 5 data from Wirtz et al. (2004). Five data from De Schamphelaere (2006) were not used by PNEC-pro because this reference was not available at the time of PNEC-pro development. The Ni-RAR contained insufficient information about the input parameters required for full BLM calculations.

Ceriodaphnia pulchella

	n	EC10 or NOEC	Geomean EC	Source	Reference
Ni-RAR	Mortality (2) Reproduction (2)	9.9-28.2 9.9-28.2	16.7	Grey Peer reviewed	(Deleebeeck *et al.*, 2006) (Deleebeeck et al., 2007b)
PNEC-pro 5	Mortality (4) Reproduction (4)	9.9-28.2 9.9-28.2	16.7	Peer reviewed	(Deleebeeck *et al.*, 2007b)
PNEC-pro 6	Reproduction (4)	7.0-27.6	13.9		

The Ni RAR and PNEC-pro databases used the same studies. PNEC-pro 6 uses EC10 values, whereas the Ni-RAR and PNEC-pro 5 selected NOECs.

Ceriodaphnia quadrangula

	n	EC10 or NOEC	Geomean EC	Source	Reference
Ni-RAR	Mortality (4) Reproduction (4)	2.0-12.7 3.5-34.9	7.4 11.0	Grey Peer reviewed	(Deleebeeck *et al.*, 2006) (Deleebeeck *et al.*, 2007b)
PNEC-pro 5	Mortality (4) Reproduction (4)	2.0-12.7 3.5-34.9	7.4 11.0	Peer reviewed	(Deleebeeck *et al.*, 2007b)
PNEC-pro 6	Reproduction (4)	2.45-33.1	8.5		

The Ni RAR and PNEC-pro databases used the same studies. PNEC-pro 6 uses EC10 values, whereas the Ni-RAR and PNEC-pro 5 selected NOECs.

Chironomus tentans

	n	EC10 or NOEC	Geomean EC	Source	Reference
Ni-RAR	Growth (4) Survival (3)	306-782 715-1196	459 781	Grey	(Stubblefield *et al.*, 2007)
PNEC-pro 5 PNEC-pro 6	Growth (5)	251-782	397	Peer reviewed	(Schlekat *et al.*, 2010)

The Ni-RAR refers to a grey publication that was not readily available. In 2010, a peer reviewed study was published which contained a full set of input parameters required for full BLM calculations. Therefore, these data were adopted in PNEC-pro 6.

Chlamidomonas sp.

	n	EC10 or NOEC	Geomean EC	Source	Reference
Ni-RAR	2	20.4-38.2	27.9	Grey	(Deleebeeck *et al.*, 2006)
PNEC-pro 5	2	8.3-27.5	15.1	Peer reviewed	(Deleebeeck *et al.*, 2009a)
PNEC-pro 6	2	26.4-45	34.5	Peer reviewed	(Deleebeeck *et al.*, 2009a)

The Ni-RAR used data from a grey study of Deleebeeck et al, whereas PNEC-pro used the data from the peer-reviewed papers from the same author. PNEC-pro 5 used the NOECs, whereas PNEC-pro 6 used the ECO values.

Chlorella sp.

	n	EC10 or NOEC	Geomean EC	Source	Reference
Ni-RAR	1	42	42	Grey	(Deleebeeck *et al.*, 2006)
PNEC-pro 5	2	64.7-90.5	76.5	Peer reviewed	(Deleebeeck *et al.*, 2009a)
PNEC-pro 6	2	64.7-90.5	76.5	Peer reviewed	(Deleebeeck *et al.*, 2009a)

Probably the peer reviewed paper of Deleebeeck contains the same tests as the grey report of the same author ((Deleebeeck *et al.*, 2006)). Although the water types are identical in the report and the peer reviewed paper, the reported EC10 values are not identical. The report is not readily available on internet, and the Ni-RAR did not describe the required BLM input parameters. Therefore, the data from the peer reviewed paper are used.

Clistorina magnifica

	n	EC10 or NOEC	Geomean EC	Source	Reference
Ni-RAR	1	66	66	Peer reviewed	(Nebeker, 1984)
PNEC-pro 5 and 6	No data				

The study of Nebeker (1984), nor the RAR, mention the values for the input parameters required for BLM calculations. Only pH, hardness and alkalinity are given. Therefore, this study is not used for PNEC-pro.

Coelastrum microporum

	n	EC10 or NOEC	Geomean EC	Source	Reference
Ni-RAR	2	41.2-51.8 (EC10)	46.2	Grey	(Deleebeeck *et al.*, 2006)
PNEC-pro 5 and 6	2	20.1-70.0 (NOECs) 38.6-100 (EC10)	31 (NOEC) 61 (EC10)	Peer reviewed	(Deleebeeck *et al.*, 2009a)

Probably the peer reviewed paper of Deleebeeck contains the same tests as the grey report of the same author (Deleebeeck *et al.*, 2006). Although the water types are identical in the report and the peer reviewed paper, the reported EC10 values are not identical. The report is not readily available on internet, and the Ni-RAR did not describe the required BLM input parameters. Therefore, the data from the peer reviewed paper are adopted in PNEC-pro.

Daphnia longispinia

	n	EC10 or NOEC	Geomean EC	Source	Reference
Ni-RAR	reproduction (2)	56.6-118	81.7	Grey	(Deleebeeck *et al.*, 2006)

	mortality (2)	26.6-29.0	27.8		
PNEC-pro 5 and 6	reproduction (2) mortality (2)	14.8-113 26.6-29.0	40.9 27.8	Peer reviewed Grey	(Deleebeeck *et al.*, 2007b) (Deleebeeck *et al.*, 2006)

Mortality is the most sensitive endpoint. PNEC-pro 5 and 6 use the same most sensitive endpoint data as the No-RAR.

Daphnia magna

	n	EC10 or NOEC	Geomean EC	Source	Reference
Ni-RAR	Growth (1) Mortality (24) Reproduction (25)	80 22.8-281 8.8-256	80 60.4 35.6	Grey and peer reviewed	(Deleebeeck et al., 2005); (Münzinger, 1994); (Munzinger, 1990); (Kuhn et al., 1989)
PNEC-pro 5	Growth (0) Mortality (7) Reproduction (25)	56.5-292 50.5-389	130 128	Grey and peer reviewed	(Kuhn *et al.*, 1989, Deleebeeck *et al.*, 2005, Deleebeeck et al., 2008)
PNEC-pro 6	Growth (0) Mortality (24) Reproduction (51)	25.2-292 4.6-389	62.0 56.7	Grey and peer reviewed	(Kuhn *et al.*, 1989, Deleebeeck *et al.*, 2005, Deleebeeck *et al.*, 2008)

Reproduction is the most sensitive endpoint. Most of the Ni-RAR data (45 out of 50) were retrieved from Deleebeeck (2005), a grey report. These data were later used by De Schamphelaere (2006) to derive the Daphnia BLM used by the RAR. This is also a grey report. The same data and models were reported in a peer reviewed paper by Deleebeeck (2008), although the reported effect concentrations do not match exactly. For PNEC pro it was decided to keep model development and model application data separate. Therefore, the test results used for BLM development were not used in PNEC pro. The remaining 32 data concern experiments with natural waters which were used for BLM validation. PNEC-pro 6 resembles the original Ni-RAR database as much as possible, and for that reason the data used for BLM development were also adopted. The required information was retrieved from the peer reviewed paper of Deleebeeck (2008). Papers of Munzinger ((Munzinger, 1990, Münzinger, 1994)) did not contain values for the required BLM input parameters, and were therefore not added to the PNEC-pro database.

Desmodesmus spinosus

	n	EC10 or NOEC	Geomean EC	Source	Reference
Ni-RAR	1	22.5	22.5	Grey	(Deleebeeck *et al.*, 2006)
PNEC-pro 5 en 6	4	3.5-43.7	19.6	Peer reviewed	(Deleebeeck *et al.*, 2009a)

Probably the peer reviewed paper of Deleebeeck (2009a) contains the same tests as the grey report of the same author ((Deleebeeck *et al.*, 2006)). Although the water types are identical in the report and the peer reviewed paper, the reported EC10 values are not identical. The report is not readily available, and the Ni-RAR does not provide the required BLM input parameters. Therefore, the data from the peer reviewed paper are used in PNEC-pro.

Gastrolphryne carolensis

	n	EC10 or NOEC	Geomean EC	Source	Reference
Ni-RAR	growth (1) malformation (2) mortality (2)	910 180-220 180-190	910 199 185	Peer reviewed	(Fort *et al.*, 2006)

PNEC-pro 5 en 6	idem				

Hyalella Azteca

	n	EC10 or NOEC	Geomean EC	Source	Reference
Ni-RAR	mortality (1)	29	29	Peer reviewed	(Keithly *et al.*, 2004)
PNEC-pro 5 en 6	idem				

Hydra littoralis

	n	**EC10 or NOEC**	**Geomean EC**	**Source**	**Reference**
Ni-RAR	**growth (1)**	**60**	**60**	**Peer reviewed**	**(Santiago-Fandino, 1983)**
PNEC-pro 5 en 6	**idem**				

Juga plicifera

	n	EC10 or NOEC	Geomean EC	Source	Reference
Ni-RAR	1	124	124	Peer reviewed	(Nebeker, 1984)
PNEC-pro 5 and 6	No data				

The study of Nebeker (1984), nor the RAR, mentions the values for the input parameters required for BLM calculations. Only pH, hardness and alkalinity are described. Therefore this study is not used for PNEC-pro.

Lemna gibba

	n	EC10 or NOEC	Geomean EC	Source	Reference
Ni-RAR	3	50-80	65.4	Peer reviewed	(Klaine et al., 2003)
PNEC-pro 5 and 6	No data				

The study of Klaine et al (2003), nor the Ni-RAR mention the values for the input parameters required for BLM calculations. Therefore, the data from this study are not used in PNEC-pro.

Lemna minor

	n	EC10 or NOEC	Geomean EC	Source	Reference
Ni-RAR	3	8.2-75	28.1	Grey	(Antunes, 2007)
PNEC-pro 5	No data				
PNEC-pro 6	5	7.5-435	37.2	Peer reviewed	(Schlekat *et al.*, 2010)

The peer reviewed study of Schlekat et al. (2010) contains different data than the grey report of Antunes (2007). The latter report was not available through the internet, and the Ni-RAR did not mention the values for the required BLM input parameters. At the time PNEC-pro was developed, the study of Schlekat et al. was not published yet. For the PNEC-pro 6 the data from the peer reviewed paper of Schlekat et al. (2010) are included in the toxicity database.

Lymnaea stagnalis

	n	EC10 or NOEC	Geomean EC	Source	Reference
Ni-RAR	Growth (3) Mortality (4)	1.4-19.5 10.2-103.8	6.9 48.8	Grey	(Stubblefield *et al.*, 2007)
PNEC-pro 5	No data				
PNEC-pro 6	Growth (5)	1.3-19.5	4.0	Peer reviewed	(Schlekat *et al.*, 2010)

The peer reviewed study of Schlekat (2010) [2] contains overlapping data with the grey report of Stubblefield (2007) (Stubblefield *et al.*, 2007). The latter report was not available on internet, and the Ni-RAR does not mention the values for the required BLM input parameters. At the time PNEC-pro was developed, the study of Schlekat (2010) was not published yet. For PNEC-pro 6, the data from the peer reviewed paper of Schlekat (2010) were included in the toxicity database.

Oncorhynchus mykiss

	n	EC10 or NOEC	Geomean EC	Source	Reference
Ni-RAR	Growth (1) Mortality (16)	134 209-1548	134 555	Peer reviewed Grey	(Nebeker et al., 1985, Deleebeeck *et al.*, 2005)
PNEC-pro 5	Mortality (5)	265-1770	615	Peer reviewed	(Deleebeeck et al., 2007a)
PNEC-pro 6	Mortality (20)	140-1770	401	Peer reviewed	(Deleebeeck *et al.*, 2007a)

Most of the Ni-RAR data were retrieved from a grey report of Deleebeeck (2005). These data were later used by De Schamphelaere (2006) to derive the Daphnia BLM used by the Ni-RAR. This is also a grey report. The same data and models were reported in a peer reviewed paper by Deleebeeck (2007a), although the reported effect concentrations do not match exactly. For PNEC-pro it was decided to keep model development and model application data separate. Therefore the test results used for BLM development were not used in PNEC-pro. The remaining 5 data concern experiments with natural waters used for validation of the BLM, and were retrieved from the peer reviewed paper of Deleebeeck (2007a). PNEC-pro 6 resembles the original Ni-RAR database as much as possible, and for that reason the data used for BLM development were also adopted. The necessary data were retrieved from the peer reviewed paper of Deleebeeck et al. (2007a). Nebeker et al. (1985) provide the only value for the most sensitive endpoint (growth), and in the Ni-RAR this value was transferred to the SSD. As a consequence, all the mortality data seem to be redundant. Since Nebeker (1984) did not report values for the required BLM input parameters, this endpoint is not included in the PNEC-pro database.

Pediastrum duplex

	n	EC10 or NOEC	Geomean EC	Source	Reference
Ni-RAR	2	20.1-28.2	23.8	Grey	(Deleebeeck *et al.*, 2006)
PNEC-pro 5	2	23.5-39.5	30.5	Peer reviewed	(Deleebeeck *et al.*, 2009a)
PNEC-pro 6	2	16.4-32.2	23.0	Peer reviewed	(Deleebeeck *et al.*, 2009a)

Probably the peer reviewed paper of Deleebeeck (Deleebeeck *et al.*, 2009a) contains the same tests as the grey report of the same author (Deleebeeck *et al.*, 2006). Although the water types are identical in the report and the peer reviewed paper, the reported EC10 values are not identical. The report is not readily available on internet, and the Ni-RAR does not provide the required BLM input parameters. Therefore, the peer reviewed paper is used for PNEC-pro 5. In PNEC-pro 6, the lowest value of NOEC and EC10 was selected, whereas initially EC10 was only selected when NOECs were not available.

Peracantha truncate

	n	EC10 or NOEC	Geomean EC	Source	Reference
NiRAR	Reproduction (2) Mortality (2)	2.5-25.8 11.3-25.8	8.0 31.9	Grey	(Deleebeeck *et al.*, 2006)
PNEC-pro 5 and 6	Reproduction (2) Mortality (2)	4. 11.3-25.89-24.7	11.0 31.9	Peer reviewed Grey	(Deleebeeck *et al.*, 2007b) (Deleebeeck *et al.*, 2006)

Pimephales promelas

	n	EC10 or NOEC	Geomean EC	Source	Reference
NiRAR	Growth (1) Mortality (2)	57 57-109	57 78.8	Grey	(Lind et al., 1978, Birge et al., 1984)
PNEC-pro 5 & 6	No data				

The studies do not report the required input parameters for BLM calculation. These data are therefore not useful in PNEC-pro.

Pseudokirchneriella subcapitata

	n	EC10 or NOEC	Geomean EC	Source	Reference
Ni-RAR	47	25.3-425	92.7	Grey	(Deleebeeck *et al.*, 2005, De Schamphelaere *et al.*, 2006)
PNEC-pro 5	14	4.9-425	81.4	Peer reviewed	(Deleebeeck *et al.*, 2005, Deleebeeck et al., 2009b)
PNEC-pro 6	49	4.9-425	83.9	Grey Peer reviewed	(Deleebeeck *et al.*, 2005, Deleebeeck *et al.*, 2009b)

Most of the Ni-RAR data were retrieved from a grey report of Deleebeeck (2005). These data were later used in a report by De Schamphelaere (2006) to derive the Daphnia BLM used by the RAR. Probably the same data are described in the peer reviewed paper of Deleebeeck (2009b), although the reported effect concentrations do not match exactly. For PNEC pro5 it was decided to keep model development and model application data separate. Therefore, the test results used for BLM development were not used in PNEC-pro 5. The remaining 14 data concern experiments with natural waters used for validation of the BLM, and were retrieved from the peer reviewed paper of Deleebeeck (2009b).

Scenedesmus accuminatus

	n	EC10 or NOEC	Geomean EC	Source	Reference
Ni-RAR	1	12.3	16.3	Grey	(Deleebeeck *et al.*, 2006)
PNEC-pro 5 and 6	2	3.1-12.3	6.2	Peer reviewed	(Deleebeeck *et al.*, 2009a)

Probably the peer reviewed paper of Deleebeeck (2009a) contains the same tests as the grey report of the same author (Deleebeeck, 2006). Although the water types are identical in the report and the peer reviewed paper, the reported EC10 values are not identical. The report is not readily available on internet, and the Ni-RAR does not provide the values for the required BLM input parameters. Therefore, the data from the peer reviewed paper are used in PNEC pro.

Simocephalus serrulatus

	n	EC10 or NOEC	Geomean EC	Source	Reference
Ni-RAR	No data				

		EC10 or NOEC	Geomean EC		
PNEC-pro 5 and 6	Reproduction (2)	6.9-45.3	17.6	Peer reviewed	(Deleebeeck *et al.*, 2007b)

The Ni-RAR does not contain toxicity data for the crustacean *Simocephalus serrulatus.* All of the species that were later published by Deleebeeck (2007) were listed in the RAR, referring to the grey reports of Deleebeeck (2006) and (De Schamphelaere *et al.*, 2006)). These reports are not readily available on internet, and the Ni-RAR does not provide the values for the required BLM input parameters. Therefore, the data from the peer reviewed paper are used in PNEC-pro.

Simocephalus vetulus

	n	EC10 or NOEC	Geomean EC	Source	Reference
Ni-RAR	Reproduction (2) Mortality (2)	9.2-28.9 9.2-28.9	16.3 16.3	Grey	(Deleebeeck *et al.*, 2006)
PNEC-pro 5	Reproduction (2) Mortality (2)	9.2-28.9 9.2-28.9	16.3 16.3	Grey Peer reviewed	(Deleebeeck *et al.*, 2006); (Deleebeeck *et al.*, 2007b)
PNEC-pro 6	Reproduction (4) Mortality (2)	9.0-23.3 9.2-28.9	14.4 16.3	Grey Peer reviewed	(Deleebeeck *et al.*, 2006); (Deleebeeck *et al.*, 2007b)

Probably the peer reviewed paper of Deleebeeck (2007b) contains the same tests as the grey report of the same author (Deleebeeck, 2006). Therefore, the PNEC-pro toxicity database combined the information on BLM parameters retrieved from the paper with the NOECs mentioned in the RAR. The report is not readily available on the internet, and the Ni-RAR does not provide the values for the required BLM input parameters. Therefore, EC10 values and water chemistry data from the peer reviewed paper are used in PNEC pro 6.

Xenopus leavis

	n	EC10 or NOEC	Geomean EC	Source	Reference
Ni-RAR	Growth (1) Malformation (3) Mortality (3)	90 84.5-260 4630-13147	90 172 6631	Peer reviewed Grey	(Hopfer et al., 1991, Fort et al. , 2004)
PNEC-pro 5	Growth (1) Malformation (3) Mortality (2)	90 84.5-260 4630-4790	90 172 4709	Peer reviewed	(Hopfer *et al.*, 1991, Fort *et al.*, 2006)
PNEC-pro 6	Growth (1) Malformation (3) Mortality (3)	90 84.5-260 4630-13147	90 172 6631	Peer reviewed	(Hopfer *et al.*, 1991, Fort *et al.*, 2006)

The Ni-RAR toxicity database is almost identical to the PNEC-pro 5 database. PNEC-pro 5 did not include one record with an LC10 of 13147 µg/L. Since mortality is not the most sensitive endpoint, it will be eliminated during SSD processing so the effect of excluding this value was nil. However, in order to harmonize the PNEC-pro database with the Ni-RAR toxicity database as much as possible, the missing value is added to the database of PNEC-pro 6.

2. Validation boundaries for the different chronic BLMs. Algae BLM Daphnia BLM Fish BLM (Table 3.2.1-12 of Ni-RAR Section Effects)

	algae	daphnia	fish
pH	5.7-7.7	5.9-8.1	5.6-8.2
DOC (mg/l)	2.5-25.8	2.5-25.8	3.8-18.4
Mg (mg/l)	1.4-19.5	1.1-20.6	1.1-16.6
Ca (mg/l)	2.4-52.2	3.0-72.7	3.8-83.0

3.   Overview of BLMs and cross-species extrapolation used for normalization of nickel toxicity data in Ni-RAR and PNEC-pro 5 and 6.

	RAR	PNEC-pro 5	PNEC-pro 6	
			pH<8.2	8.2>pH>8.7
Algae and higher aquatic plants	*P. subcapitata* [22]	*P. subcapitata* [26]	*P. subcapitata* [26]	*mean of L. minor and P. subcapitata* [23]
C. dubia	*C. dubia* [22]	*D. magna* [27]	*C. dubia* [22]	mean of *L.stagnalis, D.magna, B. calyciflorus* and *C. dubia* [23]
D. magna	*D. magna* [22]		*D. magna* [26]	
Other cladocerans, insects and amphipods	Most stringent for *D.magna* [22] and *C.dubia* [22]		Most stringent for *D.magna* [27] and *C.dubia* [22]	
Rotifers	*D. magna* [22]	*D. magna* [27]	*D. magna* [27]	
Molluscs and hydra	*C .dubia* [22]	*D. magna* [27]	*C. dubia* [22]	
Fish and amphibians	*O. mykiss* [22]	*O. mykiss* [28]	*O. mykiss* [28]	*O. mykiss* [28]

[22]   De Schamphelaere K, Van Laer L, Deleebeeck N, Muyssen B, Degryse F, Smolders E, Janssen CR. 2006. Nickel speciation and ecotoxicity in European natural surface waters: development, refinement and validation of bioavailability models. Report prepared for the nickel Producers Environmental Research Association (NiPERA). University of Ghent,, Belgium.

[23]   Nys C, Janssen CR, Van Sprang P, De Schamphelaere KAC. 2016. The effect of pH on chronic aquatic nickel toxicity is dependent on the pH itself: Extending the chronic nickel bioavailability models. *Environ Toxicol Chem* 35:1097-1106.

[26]   Deleebeeck NME, De Schamphelaere KAC, Janssen CR. 2009. Effects of Mg2+ and H+ on the toxicity of Ni2+ to unicellular green alga *Pseudokirchneriella subcapitata*: Model development and validation with surface waters. *Sci Tot Environ* 407:1901-1914.

[27]   Deleebeeck NME, De Schamphelaere KAC, Janssen C. 2008. A novel method for predicting chronic nickel bioavailability and toxicity to Daphnia magna in artificial and natural waters. *Environ Toxicol Chem* 27:2097-2107.

[28]   Deleebeeck NME, De Schamphelaere KAC, Janssen CR. 2007. A bioavailability model predicting the toxicity of nickel to rainbow trout (*Oncorhynchus mykiss*) and fathead minnow (*Pimephales promelas*) in synthetic and natural waters. *Ecotoxicol Environ Saf* 67:1-13.

4 . Overview of speciation parameters used in chemical calculations.

	WHAM default	Ni-RAR	PNECpro 5	PNECpro 6
Source				
WHAM version	6	6	6	6
Organic matter Interaction		DOC is assumed to behave like FA		
Log K Ni-FA1	1.4	1.75	1.75	1.75
Active fulvic acids	n.r.	40%		40%
			40% for toxicity database. 50% for water samples[1]	
Organic C content	n.r.	50%	50%	50%
Inorganic speciation				
NiOH (aq)	4.14	4.1	4.14	4.14
$Ni(OH)_2$ (aq)	9.0	9.0	9.0	9.0
$NiSO_4$ (aq)	2.32	2.3	2.32	2.32
$NiCO_3$ (aq)	5.78	4.57	4.568	4.568
NiCl (aq)	0.4	0.41	-0.43	-0.43
NiHCO3 (aq)	13.41	12.42	12.418	12.418
Toxicity test media speciation		Background DOC<1 mg/L was set to zero	Background DOC<1 mg/L was set to 0.3 mg/L[2]	Background DOC<1 mg/L was set to zero
Surface water Speciation		Dissolved concentrations of Zn, Al and Fe^{III} were also considered.	Dissolved concentrations of Zn and Cu were also considered.	Only dissolved concentrations of Ni were considered.

[1] The recommended active fulvic acid fraction is 40% for Ni. For water samples an average of 50% was used to enable simultaneous calculations for Cu, Ni and Zn.

[2] This concerns studies in DOC-free reconstituted waters.

[3] Fe^{3+} and Al^{3+} were allowed to form colloidal hydroxide precipitates when their solubility product was exceeded ((Cheng et al., 2005)).

5 Full-BLM calculated HC5 values for seven ecoregions using the Ni-RAR settings (numbers between brackets are the reported variances) and with the full BLMs underlying PNEC-pro 5 and 6.

HC5 (µg/L)

Ecoregion	Ni-RAR	PNEC-pro 5	PNEC-pro 6
River Teme (UK)	19.0 (10.7-29.3)	4.0	18.4
River Otter (UK)	8.1 (4.1-13.4)	14.9	6.7
River Rhine (NL)	10.8 (5.6-17.7)	12.4	10.1
River Ebro (ES)	8.7 (4.4-14.5)	14.2	8.1
Ditch (NL)	43.6 (23.7-68.6)	23.5	39.3
Neutral acidic lake (S)	12.1 (6.9-18.4)	6.0	10.7
Lake Monate (I)	7.1 (4.0-11.0)	19.1	6.2

6. Frequency of *Daphnia magna* BLM instead of the *Ceriodaphnia dubia* BLM leading to the most stringent NOEC for 9 cladocera amphipods or insects . n=number of toxicity data. In PNEC-pro 6 both BLM's are employed for each water sample, and the lowest HC5 is adopted.

BLM	n	Frequency D. magna BLM < C. dubia BLM						
		Acidic lake	Ebro	Monate	NL Ditch	Otter	Rhine	Teme
Alona affinis	1	1	0	0	1	0	0	0
Ceriodaphnia pulchella	4	4	0	0	4	0	0	0
Ceriodaphnia quadrangula	8	8	0	0	8	0	0	0
Chironomus tentans	5	5	0	2	5	0	1	2
Daphnia longispina	4	4	0	0	4	0	0	0
Hyalella azteca	1	1	0	1	1	1	1	1
Peracantha truncata	4	4	0	0	4	0	0	0
Simocephalus serrulatus	2	2	0	0	2	0	0	0
Simocephalus vetulus	6	6	0	0	6	0	0	0
Grand Total	**35**	**35**	**0**	**3**	**35**	**1**	**2**	**3**

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