Substance data sheet
- Copper -

IKSR
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a) Assignment

The Coordination Committee Rhine has decided to derive environmental quality standards for the Rhine (EQS Rhine) for the list of substances relevant for the Rhine by applying the rules of Annex V, Paragraph 1.2.3 of the Water Framework Directive (WFD).

As far as possible, these EQS Rhine are to be based on the target values developed in the ICPR so far.

The document at hand proposes environmental quality standards (EQS Rhine) for copper as substance relevant for the Rhine. These proposed environmental quality standards for the Rhine are not legally binding. Their status corresponds to the target values of the ICPR.

b) Approach when deriving EQS Rhine

When determining proposals for EQS Rhine, the method described in the *"Manual on the Methodological Framework to Derive Environmental Quality Standards for Priority Substances in Accordance with Article 16 of the Water Framework Directive (2000/60/EG)"* which is the basis for work at EU level when proposing EQS for the priority substances of WFD Annex X was applied and further developed according to the "Technical Guidance" document accomplished at the EU level.

c) Results

During its sessions on 2 and 3 July 2008 and 1 and 2 July 2009, the PLEN-CC adopted the EQS Rhine for 13 substances. The corresponding document has been published on the ICPR homepage as ICPR report no. 164. The document at hand contains an extract of the substance data sheet for copper and completes the report no. 164. Table 1 on page 2 and 3 lists the environmental quality standards (EQS Rhine) for copper as substance relevant for the Rhine.

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Table 1: Environmental quality standard overarching subjects of protection and specific environmental quality standard

Environmental quality standard (EQS) overarching subjects of protection			
Subject of protection	**Maximum concentration (PMC-EQS)**	**Monitoring value (AA-UQN)**	**Remark**
Inland surface water according to WFD (rivers and lakes)	3.6 µg/l (without correction)	2.8 µg/l dissolved (without correction)	BC: 0.5 µg/l
Other surface waters, coastal and transitional waters according to WFD	4.5 µg/l	3.5 µg/l (without correction)	BC (North Sea): between 0.14 and 0.36 µg/l
Specific environmental quality standard (EQS)			
Subject of protection	**EQS**		**Remark**
Aquatic biocoenosis (inland surface waters according to WFD)	Step I: AA-EQS = 2.8 µg/l (without correction) PMC-EQS = 3.6 µg/l (without correction) In case of excess of standard: Step II: Bioavailability is taken into account[1]		All values: dissolved concentrations BC: 0.5 µg/l
Aquatic biocoenosis (coastal and transitional waters according to WFD)	Step I: AA-EQS = 3.5 µg/l PMC-EQS = 4.5 µg/l		BC: between 0.14 and 0.36 µg/l for the North Sea Source background concentration OSPAR, 2004

[1] According to the daughter directive, monitoring data must first be compared to the derived EQS value. If measured values are in excess of this EQS, in a second step, the measured values can be compared to the EQS + background concentration. If the EQS + background concentration are exceeded, measured values may be corrected according to bioavailability. This value corrected according to the bioavailability is then compared to the EQS + background concentration.
Attention: if the bioavailability is corrected by means of the BLM method, the background concentration is already integrated into the BLM method. Measurement data corrected with the BLM must therefore be compared to the EQS values not including the background concentration.
All metal concentrations are expressed as dissolved concentrations.

Environmental quality standard (EQS) overarching subjects of protection			
Subject of protection	**Maximum concentration (PMC-EQS)**	**Monitoring value (AA-UQN)**	**Remark**
	In case of excess of standard: Step II: DOC concentration is taken into account		All values: dissolved concentrations
Sediment organism	No threshold value		
Secondary poisoning	No threshold value		Copper is an essential element, its absorption by the organism is regulated
Fish consumption	No threshold value		
Drinking water (98/83/EC)	2 mg/l		In tap water.

Legend

BLM = **B**io**l**igands **m**odel (model for calculating biological availability)

DOC = **D**issolved **o**rganic **c**arbon

BC = **B**ackground **C**oncentration

AA = **A**nnual **a**verage concentration

OSPAR = **Os**lo and **Par**is convention (OSPAR Commission)

µg = **M**icro**g**ram

EQS = **E**nvironmental **Q**uality **S**tandard

PMC = **P**ermissible **m**aximum **c**oncentration

WFD = **W**ater **F**ramework **D**irective

Annexe 1: Test results for aquatic organism

Table 1.1: Summary of the "species mean" L/EC_{50} values (total risk approach) in µg $Cu.L^{-1}$ (with geometric means per species) as used for the SSD modeling [Quelle: Wenzel (2014)].

Taxonomic group	Common name	Species	Species (Mean) Acute Value (µg Cu/L) total
Algae	Chlorophycea	*Chlorella sp. (PNG isolate)*	3.0
	Chlorophycea	*Pseudokirchneriella subcapitata*	29.1
	Chlorophycea	*Scenedesmus acutus*	29.9
	Chlorophycea	*Chlorella sp. (NT isolate)*	47.5
	Chlorophycea	*Chlamydomonas reinhardtii*	49.9
	Chlorophycea	*Scenedesmus incrassatulus*	61
	Chlorophycea	*Chlorella pyrenoidosa*	78
	Chlorophycea	*Chlorella vulgaris*	110.5
	Chlorophycea	*Scenedesmus subspicatus*	120
	Chlorophycea	*Chlorella saccharophila*	550
	Diatom	*Nitzschia linearis*	795
	Diatom	*Navicula seminulum*	805
Amphibia	Green pond frog	*Rana hexadactyla*	39
	Cope's gray tree frog	*Hyla chrysoscelis*	45
	The natterjack toad	*Epidalea calamita*	80
	Leopard frog	*Rana pipiens*	85
	Boreal toad	*Bufo boreas*	120
	Bronze frog	*Lithobates clamitans ssp. clamitans*	163
	Southern leopard frog	*Rana sphenocephala*	230
	Indian bullfrog	*Rana tigrana*	389
	African clawed frog	*Xenopus laevis*	685
Crustacea	Cladocera	*Ceriodaphnia reticulata*	17
	Cladocera	*Scapholeberis sp.*	18
	Amphipod	*Gammarus*	20.4
	Amphipod	*Hyalella azteca*	20.8
	Cladocera	*Daphnia magna*	25.7
	Cladocera	*Ceriodaphnia dubia*	26.2
	Anostraca	*Thamnocephalus platyurus.*	40
	Cladocera	*Daphnia pulicaria*	41.2
	Cladocera	*Daphnia pulex*	53
	Cladocera	*Simocephalus vetulus*	57
	Decapoda	*Macrobrachium hendersodayanus*	1750
Cyanobacteria	Cyanobacteria	*Microcystis flos-aquae*	4.5
	Cyanobacteria	*Microcystis aeruginosa*	13.9
Annelida	Worm	*Lumbriculus variega*	259.9
Bryozoa	Moss animal	*Pectinatella magnifica*	140
	Moss animal	*Lophopodella carteri*	510

Taxonomic group	Common name	Species	Species (Mean) Acute Value (µg Cu/L) total
Fish	Arctic grayling	*Thymallus arcticus*	2.6
	Northern squawfish	*Ptychocheilus orego*	20.3
	Chinook salmon	*Oncorhynchus tshaw*	32.6
	Rainbow trout	*Oncorhynchus mykiss*	34.7
	Mosquitofish	*Gambusia affinis*	56
	Coho salmon	*Oncorhynchus kisutc*	58.9
	Fountain darter	*Etheostoma rubrum*	60
	Lahontan cutthroat	*Oncorhynchus clarki henshawi*	69.3
	Guppy	*Poecilia reticulata*	69.8
	Apache trout	*Oncorhynchus*	70
	Brook trout	*Salvelinus fontenalis*	100
	Bull trout	*Salvelinus confluent*	106.9
	Cutthroat trout	*Oncorhynchus clarki*	108.3
	Fathead minnow	*Pimephales promelas <24 h*	120.8
	Pink salmon	*Oncorhynchus gorbu*	135.3
	Fathead minnow	*Pimephales promelas*	138.7
	Chiselmouth	*Acrocheilus*	143
	Shovelnose sturgeon	*Scaphirhynchus*	160
	Gila topminnow	*Poeciliposis*	160
	Sockeye salmon	*Oncorhynchus nerka*	163.0
	Bonytail chub	*Gila elegans*	200
	Greenthroat darter	*Etheostoma*	260
	Razorback sucker	*Xyrauchen texanus*	273.5
	Fantail darter	*Etheostoma flabellar*	358.2
	Northern squawfish	*Ptychocheilus orego*	427.1
	Johnny darter	*Etheostoma nigrum*	529.4
	Golden orfe	*Leuciscus idus*	565.7
	Bluegill	*Lepomis macrochirus*	1112
Insect	Midge	*Chironomus*	739
	Stonefly	*Acroneuria lycorias*	8300
Macrophyte	Macrophyte	*Lemna minor*	665.7
	Macrophyte	*Elodea nuttalli*	6000
	Macrophyte	*Callitriche platycarpa*	6000
Mollusca	Snail	*Lithoglyphus virens*	8
	Snail	*Juga plicifera*	12.8
	Mussel	*Actinonaias*	27.0
	Snail	*Physa integra*	38.9
	Mussel	*Utterbackia imbecilli*	74.8
	Snail	*Campeloma*	1673.3
Plathelminthes	Planaria	*Dugesia tigrina*	2450
Rotatoria	Rotifer	*Brachionus calyciflorus*	26

Table 1.2: Updated summary of the "species mean" NOECs (total risk approach) in µg Cu. L-1 (with geometric means and number of datapoints) as used for the SSD modelling. Information in brackets refer to the data of ECI (2008) [Quelle: Wenzel (2014)]

Organism group	**Species**	**Species mean NOEC, (µg Cu.L^{-1})**
Algae	new data *Scenedesmus acutus*; n=2; growth, from UBA PSM Database	2.75
	Pseudokirchneriella subcapitata, n=12; growth; plus 3 recent values total n=15	25.7
	Chlamydomonas reinhardtii, n=4; growth	79.8
	Chlorella vulgaris, n=19; growth; plus 2 recent values: n=21	92.3
Macrophyte	*Lemna minor*, n=1; growth	30.0
Rotifer	*Brachionus calyciflorus*; n=4; intrinsic rate of growth	33.5
Mollusca	*Campeloma decisum*, n=2; mortality;	8.0
	Juga plicifera, n=1; mortality;	6.0
	Villosa iris, n=1; mortality;	19.1
	Dreissenia polymorpha, n=2; filtration rate	18.3
Cladocera	*Ceriodaphnia dubia*, n=14; reproduction; * original data of ECI 2008 were recalculated	15.0
	Daphnia pulex, n=9; mortality	14.5
	Daphnia magna, n=11; growth, reporduction, mortality ; * original data of ECI 2008 were used plus two recent NOECs	46.5
Amphipoda	*Gammarus pulex*, n=1; reproduction;	11.0
	Hyalella azteca, n=6; mortality	50.3
Insects	*Clistoronia magnifica, n=2; reproduction/mortality;*	10.4
	Chironomus riparius, n=1; growth;	16.9
	Paratanytarsus parthenogeneticus, n=2; growth/reproduction	40.0
Fish	Catostomus commersoni; n=1; growth/mortality;	12.9
	Ictalurus punctatus, n=2; growth/mortality;	13.0
	Oncorhynchus mykiss, n=5; growth; * original data of ECI 2008 were recalculated	12.2
	Salvelinus fontinalis, n=5; growth;	15.6
	Pimephales promelas, n=4; growth;	19.7
	Oncorhynchus kisutch, n=3; mortality; * original data of ECI 2008 were recalculated	22.3
	Esox lucius; n=1; growth/mortality;	34.9
	Perca fluviatilis, n=1; growth;	39.0
	Pimephales notatus, n=2; growth;	56.2
	Noemacheilus barbatulus, n=1; mortality;	120
Amphibians	Xenopus laevis n=1, growth	40.0
	Rana pipiens n=1, growth	71.0

Annexe 2: Data on the chronic toxicity to freshwater organisms used for setting the EQS

Data on chronic toxicity tests resulting in NOEC values for freshwater algae, invertebrates and fish are summarised here below

A total of 139 individual chronic toxicity data and 27 geometric means per species chronic toxicity entries with the highest quality could be extracted from the scientific literature and databases. It appears that 36.7% of all gathered chronic toxicity data were derived from toxicity tests performed with freshwater fish, 38.8% with invertebrates and 24.5% with algae/higher plants.

Below, somewhat more detailed data are given on the selected NOEC values for freshwater algae, invertebrates and fish. Individual NOEC values seemed to range between 2.2 and 510 µg Cu/l for the total risk approach, see table below. Consistent with OECD guidelines, the average of the copper exposure concentrations and the characteristics of the test media (pH, DOC, major ions) as measured at the start and end of the test period or media renewal period were used for the assessment. For the algae, consistent with international agreements, the endpoint growth rate was used instead of the endpoint biomass. Background concentrations in the culture media and DOC concentrations, if not reported, were estimated based on available literature data – more details below.

Table 2.1: Overview of the NOEC values and physico-chemical parameters for freshwater algae/higher plants. Selected high quality Q1 NOEC values are underlined selected for the effects assessment and bioavailability normalisation. Legend see table 2.3.

Organism	Age /size of organisms	Test substance (& purity)	Exposure time	Endpoint	NOEC (µg/l)	Dose-response	Testtype	Cb (µg Cu/l)	Physico-chemical conditions	Medium	Reference
Chlamydomonas reinhardtii	Inoculum: 1,000 c/ml	$CuSO_4$ (reagent grade)	10 d	growth	**22**	/	FT	0.5*	T: 24°C; pH: 6.6; H: 25 mg/l CaCO3; DOC: 0.5 mg/l (1)	Reconstituted	Schäfer et al., 1994 (1)
Chlamydomonas reinhardtii	Inoculum: 10,000 c/ml	$CuSO_4$ (reagent grade)	3 d	growth	**178**	yes	S	0.5*	T: 20°C; pH: 6.02; H: 23 mg/l CaCO3; DOC: 9.84 mg/l	Reconstituted	De Schamphelaere et al., 2006 (2)
Chlamydomonas reinhardtii	Inoculum: 10,000 c/ml	$CuSO_4$ (reagent grade)	3 d	growth	**108**	yes	S	0.5*	T: 20°C; pH: 7.03; H: 23 mg/l CaCO3; DOC: 9.84 mg/l	Reconstituted	De Schamphelaere et al., 2006 (2)
Chlamydomonas reinhardtii	Inoculum: 10,000 c/ml	$CuSO_4$ (reagent grade)	3 d	growth	**96**	yes	S	0.5*	T: 20°C; pH: 8.11; H: 23 mg/l CaCO3; DOC: 9.84 mg/l	Reconstituted	De Schamphelaere et al., 2006 (2)
Chlorella vulgaris	Inoculum: 10,000 c/ml	$CuSO_4$ (reagent grade)	3 d	growth	**108.3**	yes	S	0.5*	T: 20°C; pH: 6.03; H: 97 mg/l CaCO3; DOC: 5.17 mg/l	Reconstituted	De Schamphelaere et al., 2006 (2)
Chlorella vulgaris	Inoculum: 10,000 c/ml	$CuSO_4$ (reagent grade)	3 d	growth	**407.4**	yes	S	0.5*	T: 20°C; pH: 6.04; H: 99 mg/l CaCO3; DOC: 15.5 mg/l	Reconstituted	De Schamphelaere et al., 2006 (2)
Chlorella vulgaris	Inoculum: 10,000 c/ml	$CuSO_4$ (reagent grade)	3 d	growth	**55.6**	yes	S	0.5*	T: 20°C; pH: 7.92; H: 388 mg/l CaCO3; DOC: 5.0 mg/l	Reconstituted	De Schamphelaere et al., 2006 (2)
Chlorella vulgaris	Inoculum: 10,000 c/ml	$CuSO_4$ (reagent grade)	3 d	growth	**36.4**	yes	S	0.5*	T: 20°C; pH: 7.04; H: 242 mg/l CaCO3; DOC: 1.5 mg/l	Reconstituted	De Schamphelaere et al., 2006 (2)
Chlorella vulgaris	Inoculum: 10,000 c/ml	$CuSO_4$ (reagent grade)	3 d	growth	**172.9**	yes	S	0.5*	T: 20°C; pH: 7.97; H: 389 mg/l CaCO3; DOC: 15.8 mg/l	Reconstituted	De Schamphelaere et al., 2006 (2)
Chlorella vulgaris	Inoculum: 10,000 c/ml	$CuSO_4$ (reagent grade)	3 d	growth	**98.9**	yes	S	0.5*	T: 20°C; pH: 7.03; H: 244 mg/l CaCO3; DOC: 10.8 mg/l	Reconstituted	De Schamphelaere et al., 2006 (2)
Chlorella vulgaris	Inoculum: 10,000 c/ml	$CuSO_4$ (reagent grade)	3 d	growth	**85.4**	yes	S	0.5*	T: 20°C; pH: 7.01; H: 486 mg/l CaCO3; DOC: 10.0 mg/l	Reconstituted	De Schamphelaere et al., 2006 (2)
Chlorella vulgaris	Inoculum: 10,000 c/ml	$CuSO_4$ (reagent grade)	3 d	growth	**161.9**	yes	S	0.5*	T: 20°C; pH: 8.75; H: 243 mg/l CaCO3; DOC: 9.9 mg/l	Reconstituted	De Schamphelaere et al., 2006 (2)
Chlorella vulgaris	Inoculum: 10,000 c/ml	$CuSO_4$ (reagent grade)	3 d	growth	**282.9**	yes	S	0.5*	T: 20°C; pH: 7.05; H: 244 mg/l CaCO3; DOC: 19.10 mg/l	Reconstituted	De Schamphelaere et al., 2006 (2)

Organism	Age /size of organisms	Test substance (& purity)	Exposure time	Endpoint	NOEC (µg/l)	Dose-response	Testtype	Cb (µg Cu/l)	Physico-chemical conditions	Medium	Reference
Chlorella vulgaris	Inoculum: 10,000 c/ml	$CuSO_4$ (reagent grade)	3 d	growth	**187.8**	yes	S	0.5*	T: 20°C; pH: 6.01; H: 389 mg/l CaCO3; DOC: 5.0 mg/l	Reconstituted	De Schamphelaere et al., 2006 (2)
Chlorella vulgaris	Inoculum: 10,000 c/ml	$CuSO_4$ (reagent grade)	3 d	growth	**510.2**	yes	S	0.5*	T: 20°C; pH: 6.05; H: 390 mg/l CaCO3; DOC: 15.2 mg/l	Reconstituted	De Schamphelaere et al., 2006 (2)
Chlorella vulgaris	Inoculum: 10,000 c/ml	$CuSO_4$ (reagent grade)	3 d	growth	**31.0**	yes	S	0.5*	T: 20°C; pH: 7.88; H: 98 mg/l CaCO3; DOC: 5.3 mg/l	Reconstituted	De Schamphelaere et al., 2006 (2)
Chlorella vulgaris	Inoculum: 10,000 c/ml	$CuSO_4$ (reagent grade)	3 d	growth	**188.0**	yes	S	0.5*	T: 20°C; pH: 7.88; H: 99 mg/l CaCO3; DOC: 15.7 mg/l	Reconstituted	De Schamphelaere et al., 2006 (2)
Chlorella vulgaris	Inoculum: 10,000 c/ml	$CuSO_4$ (reagent grade)	3 d	growth	**404.1**	yes	S	0.5*	T: 20°C; pH: 5.5; H: 244 mg/l CaCO3; DOC: 10.3 mg/l	Reconstituted	De Schamphelaere et al., 2006 (2)
Chlorella vulgaris	Inoculum: 10,000 c/ml	$CuSO_4$ (reagent grade)	3 d	growth	**158.7**	yes	S	0.5*	T: 20°C; pH: 7.07; H: 25 mg/l CaCO3; DOC: 10.3 mg/l	Reconstituted	De Schamphelaere et al., 2006 (2)
Chlorella vulgaris	Inoculum: 10,000 c/ml	$CuSO_4$ (reagent grade)	3 d	growth	**83.9**	yes	S	0.5*	T: 20°C; pH: 7.03; H: 244 mg/l CaCO3; DOC: 10.8 mg/l	Reconstituted	De Schamphelaere et al., 2006 (2)
Chlorella vulgaris	Inoculum: 10,000 c/ml	$CuSO_4$ (reagent grade)	3 d	growth	**132.3**	yes	S	0.5*	T: 20°C; pH: 7.04; H: 246 mg/l CaCO3; DOC: 10.2 mg/l	Reconstituted	De Schamphelaere et al., 2006 (2)
Chlorella sp. (PNG isolate)		CuSO4 (analytical grade)	3 d	growth	**2.3**		S			artificial	Levy et al. 2009
Chlorella sp. (NT isolate) (pH 5.7)		CuSO4 (analytical grade)	3 d	growth	**4**		S			artificial	Levy et al. 2009
Pseudokirchneriella subcapitata	Inoculum: 10,000 c/ml	$CuSO_4$ (reagent grade)	3 d	growth	**52.9**	yes	S	0.5*	T: 20°C; pH: 6.74; H: 10.0 mg/l CaCO3; DOC: 2.72 mg/l	Lake	Heijerick et al., 2002 (3)
Pseudokirchneriella subcapitata	Inoculum: 10,000 c/ml	$CuSO_4$ (reagent grade)	3 d	growth	**61.8**	yes	S	0.5*	T: 20°C; pH: 7.0; H: 12.4 mg/l CaCO3; DOC: 2.34 mg/l	Lake	Heijerick et al., 2002 (3)
Pseudokirchneriella subcapitata	Inoculum: 10,000 c/ml	$CuSO_4$ (reagent grade)	3 d	growth	**94.7**	yes	S	0.5*	T: 20°C; pH: 6.14; H: 7.9 mg/l CaCO3; DOC: 12 mg/l	Lake	Heijerick et al., 2002 (3)
Pseudokirchneriella subcapitata	Inoculum: 10,000 c/ml	$CuSO_4$ (reagent grade)	3 d	growth	**17.9**	yes	S	0.5*	T 20°C; pH: 7.66; H: 48.7 mg/l CaCO3; DOC: 2.52 mg/l	Lake	Heijerick et al., 2002 (3)

Organism	Age /size of organisms	Test substance (& purity)	Exposure time	Endpoint	NOEC (µg/l)	Dose-response	Testtype	Cb (µg Cu/l)	Physico-chemical conditions	Medium	Reference
Pseudokirchneriella subcapitata	Inoculum: 10,000 c/ml	$CuSO_4$ (reagent grade)	3 d	growth	**49**	yes	S	0.5*	T: 20°C; pH: 8.0; H: 220 mg/l CaCO3; DOC: 6.42 mg/l	Lake	Heijerick et al., 2002 (3)
Pseudokirchneriella subcapitata	Inoculum: 10,000 c/ml	$CuSO_4$ (reagent grade)	3 d	growth	**35.4**	yes	S	0.5*	T: 20°C; pH: 7.84; H: 238 mg/l CaCO3; DOC: 8.24 mg/l	Lake	Heijerick et al., 2002 (3)
Pseudokirchneriella subcapitata	Inoculum: 10,000 c/ml	$CuSO_4$ (reagent grade)	3 d	growth	**23.1**	yes	S	0.5*	T: 20°C; pH: 7.93; H: 191 mg/l CaCO3; DOC: 1.99 mg/l	River	Heijerick et al., 2002 (3)
Pseudokirchneriella subcapitata	Inoculum: 10,000 c/ml	$CuSO_4$ (reagent grade)	3 d	growth	**19.3**	yes	S	0.5*	T: 20°C; pH: 7.93; H: 191 mg/l CaCO3; DOC: 1.99 mg/l	River	Heijerick et al., 2002 (3)
Pseudokirchneriella subcapitata	Inoculum: 10,000 c/ml	$CuSO_4$ (reagent grade)	3 d	growth	**56.4**	yes	S	0.5*	T: 20°C; pH: 7.69; H: 132 mg/l CaCO3; DOC: 6.13 mg/l	River	Heijerick et al., 2002 (3)
Pseudokirchneriella subcapitata	Inoculum: 10,000 c/ml	$CuSO_4$ (reagent grade)	3 d	growth	**164**	yes	S	0.5*	T: 20°C; pH: 7.84; H: 166 mg/l CaCO3; DOC: 17.8 mg/l	Lake	Heijerick et al., 2002 (3)
Pseudokirchneriella subcapitata	Inoculum: 10,000 c/ml	$CuSO_4$ (reagent grade)	3 d	growth	**65.5**	yes	S	0.5*	T: 20°C; pH: 7.35; H: 134 mg/l CaCO3; DOC: 20.4 mg/l	Lake	Heijerick et al., 2002 (3)
Pseudokirchneriella subcapitata	Inoculum: 10,000 c/ml	$CuSO_4$ (reagent grade)	3 d	growth	**15.7**	yes	S	0.5*	T: 20°C; pH: 8.16; H: 169 mg/l CaCO3; DOC: 1.7 mg/l	Lake	Heijerick et al., 2002 (3)
Pseudokirchneriella subcapitata		CuSO4 (analytical grade)	3 d	growth	**0.3**		S		Der Wert ist nicht valide. Die analytisch bestimmt Konzentrationen weichen erheblich von den Nominalwerten ab. Es wurden Angaben unterhalb der angegeben Nachweisgrenze verwendet.Daher wird der Wert nicht für die UQN Ableitung herangezogen.	artificial	Levy et al. 2009
Pseudokirchneriella subcapitata	Inoculum: 10,000 c/ml	CuSO4 (reagent grade)	3 d	growth	**14**	yes	S			OECD 201 medium	Aruoja et al. 2009

Organism	Age /size of organisms	Test substance (& purity)	Exposure time	Endpoint	NOEC (µg/l)	Dose-response	Testtype	Cb (µg Cu/l)	Physico-chemical conditions	Medium	Reference
Pseudokirchneriella subcapitata		Cu 2+	3 d	growth	**8.4**		S				Kusel-Fetzmann Fetzmann and Latif, 1989
Scenedesmus acutus		No information	3 d	growth	**2.3**		S			artificial	Kusel-Fetzmann Fetzmann and Latif, 1989
Scenedesmus acutus		No Information	3 d	growth	**3.3**		S			artificial	Kusel-Fetzmann Fetzmann and Latif, 1989
Lemna minor	Double fronded colonies	$CuSO_4$ (analytical grade)	7 d	growth	**30**	/	S	0.5*	T: 25°C; pH: 6.5; H : 26.8 mg/l CaCO3; DOC: 0.5 mg/l (1)	artificial	Teisseire et al., 1998 (4)

1. Schäfer *et al.*, 1994

Comments: Background Cu concentrations in control water (artificial water) are not reported,

- Mean alkalinity and hardness of culture media (OECD medium) for *S. subspicatus* is 1.22 10^{-4} M/l $CaCl_2$, 6.1 10^{-5} M/l $MgSO_4$, 5.9 10^{-5} M/l $MgCl_2$ (total hardness of 25 mg/l $CaCO_3$); pH 8,
- Mean alkalinity and hardness of culture media (SAG medium) for *C. reinhardtii* (static test) is 18 mg/l $CaCl_2$, 29.5 mg/l $MgSO_4$, (total hardness of 25 mg/l $CaCO_3$); pH 8,
- Mean hardness of culture media for *C. reinhardtii* (flow through test) is 18 mg/l $CaCl_2$ 29.5 mg/l $MgSO_4$ (total hardness of 25 mg/l $CaCO_3$); pH is 6.2,
- DOC concentration was estimated as 0.5 mg/l for reconstituted waters,
- Statistics are reported,
- Cu concentrations tested are not reported,
- Dose responses are not reported,
- Reported NOEC data for *C. reinhardtii* are 5 (static), **22** (flow-through) µg/l Cu (growth - biomass) and 636 µg/l Cu (photosynthesis).
- Reported NOEC data for *S. subspicatus* are 56 (static) µg/l (growth) and 41 µg/l Cu (photosynthesis)
- Only the data from the flow-through experiment were retained. The rejected data : nominal concentrations reported in static exposure system.

2. De Schamphelaere et al.,2006

Comments:

- All tests were performed according to the OECD guidelines (N°201 for *Chlorella vulgaris* and *Chlamydomonas*),
- Background Cu concentrations in control water (reconstituted) are not reported,
- Mean hardness of testmedia was 23 mg/l $CaCO_3$ for the test with *Chlamydomonas reinhardtii* and varied between 25 and 486 mg/l $CaCO_3$ for *Chlorella vulgaris*,
- Reported pH value varied between 6.0 and 8.0 for the *Chlamydomonas reinhardtii* tests and between 5.5 and 8.75 for the *Chlorella vulgaris* tests,
- DOC reported between 1.5 and 19.1 mg/l,
- Statistics are reported,
- Dose response curve are reported,
- Cu concentration tested (5 concentrations and 1 control),
- Reliable NOEC (3 days of exposure) values for *Chlamydomonas reinhardtii* are (endpoint growth - biomass): **178, 108, 96** µg/l Cu,
- Reliable NOEC (3 days of exposure) values for *C. vulgaris* are (endpoint growth) **108.3, 407.4, 55.6, 36.4, 172.9, 98.9, 85.4, 161.9, 282.9, 187.8, 510.2, 31, 188, 404.1, 158.7, 83.9 and 132.3** µg/l Cu.

3. Heijerick et al., 2002

Comments:

- All tests were performed according to the OECD guidelines (N°201 for *Pseudokirchneriella subcapitata)*,
- Background Cu concentrations in control water (reconstituted) are not reported,
- Mean hardness of testmedia varied between 7.9 and 238 mg/l $CaCO_3$ for the algae tests,
- Natural DOC extracted from rivers and lakes (between 1.99 and 20.4 mg/l),
- Reported pH value varied between 6.14 and 8.16 for the algae tests,
- Statistics are reported,
- Dose response curve are reported,
- Cu concentration tested (5 concentrations and 1 control),
- Reliable NOEC (3 days of exposure) values for *P. subcapitata* are (endpoint growth - biomass): **52.9, 61.8, 94.7, 17.9, 49, 35.4, 23.1, 19.3, 56.4, 164, 65.5 and 15.7** µg/l Cu.

4. Teisseire et al., 1998

Comments:

- Background Cu concentrations in control water (artificial growth medium) are not reported,
- Mean hardness of testmedia is 26.8 mg/l $CaCO_3$,
- DOC concentration was assumed to be 0.5 mg/l,
- Reported pH value is 6.5,
- Statistics are reported,
- Dose response curve are reported,
- Cu concentration tested (5 concentrations and 1 control),
- Reliable NOEC (7 days of exposure) value for *Lemna minor* is (endpoint growth): **30** µg/l Cu.

Table 2.2: Overview of the NOEC values and physico-chemical parameters for freshwater invertebrates. Selected NOEC high quality Q1 values are underlined selected for the effects assessment and bioavailability normalisation. Legend see table 2.3.

Organism	Age/size of organisms	Test substance (& purity)	Exposure time	Endpoint	NOEC (µg/l)	Dose-response	Testtype	Cb (µg Cu/l)	Physico-chemical conditions	Medium	Reference
Ceriodaphnia dubia	neonates (< 24 h)	$CuSO_4$ (reagent grade)	7 d	reproduction	**10**	/	R	0.5*	T: 23°C; pH: 7.6; H: 85 mg/l CaCO3; DOC: 0.5 mg/l (1)	Reconstitued	Cerda & Olive, 1993 (5)
Ceriodaphnia dubia	neonates (< 24 h)	$CuSO_4$ (reagent grade)	7 d	mortality	**20**	/	R	0.5*	T: 23°C; pH: 7.6; H: 85 mg/l CaCO3; DOC: 0.5 mg/l (1)	Reconstitued	Cerda & Olive, 1993 (5)
Ceriodaphnia dubia	neonates (< 24 h)	Not reported (AA standard)	7 d	reproduction	**10**	yes	S	1.5*	T: 25°C; pH: 9.0; H: 98 mg/l CaCO3; DOC: 2.9 mg/l (2)	River (New River)	Belanger & Cherry, 1990 (6)
Ceriodaphnia dubia	neonates (< 24 h)	Not reported (AA standard)	7 d	reproduction	**20**	yes	S	1.5*	T: 25°C; pH: 8.0; H: 114 mg/l CaCO3; DOC: 2 mg/l (3)	River (Amy Bayou)	Belanger & Cherry, 1990 (6)
Ceriodaphnia dubia	neonates (< 24 h)	Not reported (AA standard)	7 d	reproduction	**20**	yes	S	1.5*	T: 25°C; pH: 9.0; H: 114 mg/l CaCO3; DOC: 2 mg/l (3)	River (Amy Bayou)	Belanger & Cherry, 1990 (6)

Organism	Age/size of organisms	Test substance (& purity)	Exposure time	Endpoint	NOEC (µg/l)	Dose-response	Testtype	Cb (µg Cu/l)	Physico-chemical conditions	Medium	Reference
Ceriodaphnia dubia	neonates (< 24 h)	Not reported (AA standard)	7 d	reproduction	**20**	yes	S	1.5*	T: 25°C; pH: 6.0; H: 182 mg/l CaCO3; DOC: 3 mg/l (4)	River (Clinch River)	Belanger & Cherry, 1990 (6)
Ceriodaphnia dubia	neonates (< 8 h)	Not reported (not reported)	7 d	mortality	**19**	/	S	/	T: 25°C; pH: 7.0; H: 22 mg/l CaCO3; DOC: 2 mg/l (3)	River	Jop et al., 1995 (7)
Ceriodaphnia dubia	neonates (< 8 h)	Not reported (not reported)	7 d	mortality	**4**	/	S	/	T: 25°C; pH: 6.95; H: 20 mg/l CaCO3; DOC: 0.5 mg/l (1)	Reconstituted	Jop et al., 1995 (7)
Ceriodaphnia dubia	neonates (< 24 h)	$Cu(NO_3)_2$ (reagent grade)	7 d	mortality	**122**	yes	R	3.4	T: 25°C; pH: 8.25; H: 100 mg/l CaCO3; DOC: 5.7 mg/l (5)	River (Lester River)	Spehar & Fiandt, 1985 (8)
Ceriodaphnia dubia	neonates (2-8 h)	Not reported (AA standard)	7 d	reproduction	**6.3**	yes	S	1.5	T: 25°C; pH: 8.15; H: 94 mg/l CaCO3; DOC: 2.9 mg/l (2)	River (New River)	Belanger et al., 1989 (9)
Ceriodaphnia dubia	neonates (2-8 h)	Not reported (AA standard)	7 d	reproduction	**24.1**	yes	S	4.7	T: 25°C; pH: 8.31; H: 179 mg/l CaCO3; DOC: 3 mg/l (4)	River (Clinch River)	Belanger et al., 1989 (9)

Organism	Age/size of organisms	Test substance (& purity)	Exposure time	Endpoint	NOEC (µg/l)	Dose-response	Testtype	Cb (µg Cu/l)	Physico-chemical conditions	Medium	Reference
Ceriodaphnia dubia	neonates (< 8 h)	Not reported (not reported)	7 d	reproduction	**4**	/	S	/	T: 25°C; pH: 6.3-7.6; H: 20 mg/l CaCO3; DOC: 0.5 mg/l (1)	Reconstituted	Jop et al., 1995 (7)
Ceriodaphnia dubia	neonates (< 8 h)	Not reported (not reported)	7 d	reproduction	**10**	/	S	/	T: 25°C; pH: 6.6-7.4; H: 22 mg/l CaCO3; DOC: 2 mg/l (3)	River	Jop et al., 1995 (7)
Ceriodaphnia dubia	neonates (< 24 h)	$Cu(NO_3)_2$ (reagent grade)	7 d	reproduction	**31.6**	yes	S	3.4	T: 25°C; pH: 8.25; H: 100 mg/l CaCO3; DOC: 5.7 mg/l (5)	River (Lester River)	Spehar & Fiandt, 1985 (8)
Daphnia magna	neonates	$CuCl_2$ (purity >99%)	21 d	growth	**12.6**	yes	R	2.6	T: 20°C; pH: 8.1; H: 225 mg/l CaCO3; DOC: 2 mg/l (3)	Lake (Lake Ijssel)	Van Leeuwen et al., 1988 (10)
Daphnia magna	neonates	$CuCl_2$ (purity >99%)	21 d	mortality	**36.8**	yes	R	2.6	T: 20°C; pH: 8.1; H: 225 mg/l CaCO3; DOC: 2 mg/l (3)	Lake (Lake Ijssel)	Van Leeuwen et al., 1988 (10)

Organism	Age/size of organisms	Test substance (& purity)	Exposure time	Endpoint	NOEC (µg/l)	Dose-response	Testtype	Cb (µg Cu/l)	Physico-chemical conditions	Medium	Reference
Daphnia magna	neonates	$CuCl_2$ (purity >99%)	21 d	population growth	**36.8**	/	FT	2.6	T: 20°C; pH: 8.1; H: 225 mg/l CaCO3; DOC: 2 mg/l (3)	Lake (Lake Ijssel)	Van Leeuwen et al., 1988 (10)
Daphnia magna	neonates	$CuSO_4$ (reagent grade)	21 d	reproduction	**28**	yes	R	0.5*	T: 20°C; pH: 6.31; H: 10 mg/l CaCO3; DOC: 2.72 mg/l	Lake	Heijerick et al., 2002 (11)
Daphnia magna	neonates	$CuSO_4$ (reagent grade)	21 d	reproduction	**21.5**	yes	R	0.5*	T: 20°C; pH: 6.1; H: 12.4 mg/l CaCO3; DOC: 2.34 mg/l	Lake	Heijerick et al., 2002 (11)
Daphnia magna	neonates	$CuSO_4$ (reagent grade)	21 d	reproduction	**71.4**	yes	R	0.5*	T: 20°C; pH: 8.3; H: 238 mg/l CaCO3; DOC: 8.24 mg/l	Lake	Heijerick et al., 2002 (11)
Daphnia magna	neonates	$CuSO_4$ (reagent grade)	21 d	reproduction	**68.8**	yes	R	0.5*	T: 20°C; pH: 8.06; H: 191 mg/l CaCO3; DOC: 1.99 mg/l	River	Heijerick et al., 2002 (11)
Daphnia magna	neonates	$CuSO_4$ (reagent grade)	21 d	reproduction	**106**	yes	R	0.5*	T: 20°C; pH: 7.55; H: 132 mg/l CaCO3; DOC: 6.13 mg/l	River	Heijerick et al., 2002 (11)

Organism	Age/size of organisms	Test substance (& purity)	Exposure time	Endpoint	NOEC (µg/l)	Dose-response	Testtype	Cb (µg Cu/l)	Physico-chemical conditions	Medium	Reference
Daphnia magna	neonates	$CuSO_4$ (reagent grade)	21 d	reproduction	**181**	yes	R	0.5*	T: 20°C; pH: 7.5; H: 134 mg/l CaCO3; DOC: 20.4 mg/l	Lake	Heijerick et al., 2002 (11)
Daphnia magna	neonates	$CuCl_2$ (reagent grade)	21 d	reproduction	**75**	yes	R		T: 20°C; pH: 7.6; H: 200 mg/l CaCO3; DOC: 4 mg/l	Reconstituted + DOC	Muyssen and Janssen, 2007
Daphnia magna	neonates	Cu-oxychloride	21 d	reproduction	**30**	yes	R			Reconstituted	UBA PSM database (Study DRE73981)
Daphnia pulex	neonates (< 24 h)	$CuSO_4$ (reagent grade)	42 d	mortality	**4**	yes	R	0.5*	T: 20°C; pH: 8.6; H: 57.5 mg/l CaCO3; DOC: 0.1 mg/l (6)	Deionized reconstituted	Winner, 1985 (12)
Daphnia pulex	neonates (< 24 h)	$CuSO_4$ (reagent grade)	42 d	mortality	**20**	yes	R	0.5*	T: 20°C; pH: 8.5; H: 57.5 mg/l CaCO3; DOC: 0.475 mg/l (6)	Deionized reconstituted + DOC	Winner, 1985 (12)
Daphnia pulex	neonates (< 24 h)	$CuSO_4$ (reagent grade)	42 d	mortality	**30**	yes	R	0.5*	T: 20°C; pH: 8.7; H: 57.5 mg/l CaCO3; DOC: 0.85 mg/l (6)	Deionized reconstituted + DOC	Winner, 1985 (12)

Organism	Age/size of organisms	Test substance (& purity)	Exposure time	Endpoint	NOEC (µg/l)	Dose-response	Testtype	Cb (µg Cu/l)	Physico-chemical conditions	Medium	Reference
Daphnia pulex	neonates (< 24 h)	$CuSO_4$ (reagent grade)	42 d	mortality	**5**	yes	R	0.5*	T: 20°C; pH: 8.7; H:115 mg/l CaCO3; DOC: 0.1 mg/l (6)	Deionized reconstituted	Winner, 1985 (12)
Daphnia pulex	neonates (< 24 h)	$CuSO_4$ (reagent grade)	42 d	mortality	**20**	yes	R	0.5*	T: 20°C; pH: 8.55; H: 115 mg/l CaCO3; DOC: 0.475 mg/l (6)	Deionized reconstituted + DOC	Winner, 1985 (12)
Daphnia pulex	neonates (< 24 h)	$CuSO_4$ (reagent grade)	42 d	mortality	**40**	yes	R	0.5*	T: 20°C; pH: 8.55; H:115 mg/l CaCO3; DOC: 0.85 mg/l (6)	Deionized reconstituted + DOC	Winner, 1985 (12)
Daphnia pulex	neonates (< 24 h)	$CuSO_4$ (reagent grade)	42 d	mortality	**10**	yes	R	0.5*	T: 20°C; pH: 8.55; H: 230 mg/l CaCO3; DOC: 0.175 mg/l (6)	Deionized reconstituted + DOC	Winner, 1985 (12)
Daphnia pulex	neonates (< 24 h)	$CuSO_4$ (reagent grade)	42 d	mortality	**15**	yes	R	0.5*	T: 20°C; pH: 8.6; H: 230 mg/l CaCO3; DOC: 0.475 mg/l (6)	Deionized reconstituted + DOC	Winner, 1985 (12)
Daphnia pulex	neonates (< 24 h)	$CuSO_4$ (reagent grade)	42 d	mortality	**20**	yes	R	0.5*	T: 20°C; pH: 8.6; H: 230 mg/l CaCO3; DOC: 0.85 mg/l (6)	Deionized reconstituted + DOC	Winner, 1985 (12)

Organism	Age/size of organisms	Test substance (& purity)	Exposure time	Endpoint	NOEC (µg/l)	Dose-response	Testtype	Cb (µg Cu/l)	Physico-chemical conditions	Medium	Reference
Brachionus calyciflorus	neonates (< 2 h)	$CuSO_4$ (reagent grade)	2 d	reproduction	**8.2**	yes	S	0.3	T: 25°C; pH: 6.0; H: 100 mg/l CaCO3; DOC: 4.9 mg/l	Reconstituted	De Schamphelaere et al., 2006 (13)
Brachionus calyciflorus	neonates (< 2 h)	$CuSO_4$ (reagent grade)	2 d	reproduction	**31.2**	yes	S	0.3	T: 25°C; pH: 6.0; H: 100 mg/l CaCO3; DOC: 14.5 mg/l	Reconstituted	De Schamphelaere et al., 2006 (13)
Brachionus calyciflorus	neonates (< 2 h)	$CuSO_4$ (reagent grade)	2 d	reproduction	**47.8**	yes	S	0.3	T: 25°C; pH: 7.8; H: 100 mg/l CaCO3; DOC: 4.84 mg/l	Reconstituted	De Schamphelaere et al., 2006 (13)
Brachionus calyciflorus	neonates (< 2 h)	$CuSO_4$ (reagent grade)	2 d	reproduction	**103**	yes	S	0.3	T: 25°C; pH: 7.8; H: 100 mg/l CaCO3; DOC: 14.7 mg/l	Reconstituted	De Schamphelaere et al., 2006 (13)
Gammarus pulex	mixed sizes (1.5-14 mm)	$CuSO_4$ (not reported)	100 d	population response	**11**	yes	FT	2.6	T: 11°C; pH: 8.0; H: 103 mg/l CaCO3; DOC: 1 mg/l (7)	Tap	Maund et al., 1992 (14)
Hyalella azteca	2 - 3 weeks old	$CuSO_4$ (not reported)	10 d	mortality	**50**	yes	S	/	T: 20°C; pH: 7.65; H: 36 mg/l CaCO3; DOC: 1 mg/l (8)	Spring	Deaver & Rodgers, 1996 (15)

Organism	Age/size of organisms	Test substance (& purity)	Exposure time	Endpoint	NOEC (µg/l)	Dose-response	Testtype	Cb (µg Cu/l)	Physico-chemical conditions	Medium	Reference
Hyalella azteca	2 - 3 weeks old	$CuSO_4$ (not reported)	10 d	mortality	**50**	yes	S	/	T: 20°C; pH: 7.8; H: 50 mg/l CaCO3; DOC: 1 mg/l (8)	Spring	Deaver & Rodgers, 1996 (15)
Hyalella azteca	2 - 3 weeks old	$CuSO_4$ (not reported)	10 d	mortality	**82**	yes	S	/	T: 20°C; pH: 8.05; H: 64 mg/l CaCO3; DOC: 1 mg/l (8)	Spring	Deaver & Rodgers, 1996 (15)
Hyalella azteca	2 - 3 weeks old	$CuSO_4$ (not reported)	10 d	mortality	**82**	yes	S	/	T: 20°C; pH: 7.5; H: 22 mg/l CaCO3; DOC: 1 mg/L (8)	Spring	Deaver & Rodgers, 1996 (15)
Hyalella azteca	2 - 3 weeks old	$CuSO_4$ (not reported)	10 d	mortality	**30**	yes	S	/	T: 20°C; pH: 6.95; H: <10 mg/l CaCO3; DOC: 1 mg/l (8)	Spring	Deaver & Rodgers, 1996 (15)
Hyalella azteca	<7 days old	Not reported (not reported)	35 d	mortality	**32**	yes	R	3.0	T: 22°C; pH: 7.6; H: 128 mg/l CaCO3; DOC: 1 mg/l (7)	Tap	Othman & Pascoe, 2002 (16)
Chironomus riparius	eggs (< 12 h)	$CuSO_4$ (not reported)	10 d	growth	**16.9**	yes	R	0.5*	T: 20°C; pH: 6.8; H: 151 mg/l CaCO3; DOC: 0.5 mg/l (1)	Reconstituted	Taylor et al., 1991 (17)

Organism	Age/size of organisms	Test substance (& purity)	Exposure time	Endpoint	NOEC (µg/l)	Dose-response	Testtype	Cb (µg Cu/l)	Physico-chemical conditions	Medium	Reference
Clistoronia magnifica	larvae 1st generation	$CuCl_2$ (reagent grade)	240 d	Life cycle	**8.3**	yes	FT	/	T: 15°C; pH: 7.3; H: 26 mg/l CaCO3; DOC: 1.3 mg/l (9)	Well	Nebeker et al., 1984 (18)
Clistoronia magnifica	larvae-2nd generation	$CuCl_2$ (reagent grade)	240 d	Life cycle	**13**	yes	FT	/	T: 15°C; pH: 7.3; H: 26 mg/l CaCO3; DOC: 1.3 mg/l (9)	Well	Nebeker et al., 1984 (18)
Paratanytarsus parthenogeneticus	larvae (7 days old)	$CuSO_4$ (not reported)	16 d	growth	**40**	yes	/	0.5*	T: 23°C; pH: 6.9; H: 25 mg/l CaCO3; DOC: 0.5 mg/l (1)	Reconstituted	Hatakeyama & Yasuno, 1981 (19)
Paratanytarsus parthenogeneticus	larvae (7 days old)	$CuSO_4$ (not reported)	16 d	reproduction	**40**	yes	/	0.5*	T: 23°C; pH: 6.9; H: 25 mg/l CaCO3; DOC: 0.5 mg/l (1)	Reconstituted	Hatakeyama & Yasuno, 1981 (19)
Dreissenia polymorpha	18-22 mm	$CuCl_2$ (not reported)	63-77 d	Filtration rate	**13**	/	S	3.0	T: 15°C; pH: 7.9; H: 150 mg/l CaCO3; DOC: <7.34 mg/l (10)	Lake (Lake Markermeer)	Kraak et al., 1994 (20)
Dreissenia polymorpha	18-22 mm	$CuSO_4$ (not reported)	27 d	Filtration rate	**21**	yes	R	/	T: 13.4°C; pH: 7.8; H: 296 mg/l CaCO3; DOC: 1.0 mg/l (7)	Tap	Mersch et al., 1993 (21)

Organism	Age/size of organisms	Test substance (& purity)	Exposure time	Endpoint	NOEC (µg/l)	Dose-response	Testtype	Cb (µg Cu/l)	Physico-chemical conditions	Medium	Reference
Villosa iris	glochidia	$CuSO_4$ (not reported)	30 d	mortality	**19.1**	yes	FT	3.2	T: 20.8°C; pH: 8.4; H: 152 mg/l CaCO3; DOC: 3.0 mg/l (4)	River (Clinch River)	Jacobson et al., 1997 (22)
Campeloma decisum	11 to 27 mm snail	$CuSO_4$ (ACS grade)	42 d	mortality	**8**	yes	FT	1.9	T: 15°C; pH: 8.15; H: 44.9 mg/l CaCO3; DOC: 1 mg/l (7)	Tap	Arthur & Leonard, 1970 (23)
Campeloma decisum	11 to 27 mm snail	$CuSO_4$ (ACS grade)	42 d	mortality	**8**	yes	FT	1.9	T: 15°C; pH: 8.15; H: 44.9 mg/l CaCO3; DOC: 1 mg/l (7)	Tap	Arthur & Leonard, 1970 (23)
Juga plicifera	mature	$CuCl_2$ (reagent grade)	30 d	mortality	**6**	/	FT	0.5*	T: 15°C; pH: 7.1; H: 21mg/l CaCO3; DOC: 1.3 mg/l (9)	Well	Nebeker et al., 1986 (24)

5. Cerda & Olive, 1993

Comments:

- Background Cu concentration in control water (reconstituted water) was not reported,
- Mean hardness of testmedia is 85 mg/l, mean alkalinity of testmedia is 62 mg/l and mean reported pH value is 7.6,
- DOC concentration was estimated from the values reported Santore et al. (2002), i.e. 0.5 mg/l for reconstituted waters,
- Statistics are reported,
- 11 Cu concentration tested, between 5 and 100 µg/l Cu,
- No dose response curve was given,
- Effects of 4 different diets was tested: 1. Selenastrum, 2. Chlamydomonas, 3. YCTF+Selenastrum and 4. YCTF. Only the diet YCTF+Selenastrum fulfilled in 100% of the cases the validity criteria of >80% survival and 15 young/female.
- Reliable NOEC data for *C. dubia* is **20** µg/l (survival) and **10** µg/l (reproduction)

6. Belanger and Cherry, 1990

Comments:

- Background Cu concentrations in control water are reported (< 3 µg/l Cu),
- Information concerning the culture water (i.e. New river and Clinch river),
- Origin of the fish: US EPA Duluth laboratory stock,
- Mean alkalinity and hardness of New River is 74.2 and 97.6 mg/l $CaCO_3$,
- Mean alkalinity and hardness of Clinch River is 144.3 and 182.0 mg/l $CaCO_3$,
- Mean alkalinity and hardness of Amy Bayou is 121.9 and 113.6 mg/l $CaCO_3$,
- Reported pH value is 8.12, 8.29 and 8.27 for New river, Clinch river and Amy Bayou,
- DOC concentration of 3.0 mg/l was estimated from the reported TOC concentration in the Clinch and New River water (i.e. respectively 3.7/3.65 mg/l; source: USGS database), and from Santore et al. (2002) for the Amy Bayou River with unknown DOC concentration (i.e. 2.0 mg/l),
- Statistics are reported,
- 2 Cu concentration tested (10- 40 µg/l Cu) and 1 control,
- Dose response curve is reported,
- Reliable NOEC value for *C. dubia* on reproduction is **10** (New river at pH 9), **20** and **20** (Amy Bayou at pH 8 and 9) and **20** (Clinch river at pH 6) µg/l Cu.

7. Jop et al., 1995

Comments:

- Background Cu concentrations in control waters are reported: 8.4 µg/l (river water) & < 1 µg/l (reconstitued water),
- Mean hardness of testmedia is 20 mg/l (reconstituted water) and between 16 and 28 mg/l (river water) $CaCO_3$,
- Mean alkalinity of testmedia is 19 mg/l (reconstituted water) and 13 mg/l (river water) $CaCO_3$,
- Mean reported pH value is 7.0 for both dilution waters,
- DOC concentration was estimated from the values reported Santore et al. (2002), i.e. 2.0 mg/l for natural river waters and 0.5 mg/l for reconstituted waters,
- Statistics are reported,
- 4 Cu concentration tested, 1- 64 µg/l Cu for *Ceriodaphnia dubia*,
- No dose response was given,
- Reliable reported NOEC data for *C. dubia* is **4** µg/l (Cu survival and reproduction in reconstituted water), **19** µg/l Cu (survival in river water) and **10** µg/l Cu (reproduction in river water),

8. Spehar and Fiandt, 1985 (EPA document)

Comments:

- Background Cu concentrations in control waters are reported, 3.4 µg/l for the Lester water with *C. dubia*,
- All the organisms were cultured in their respective water before they were tested,
- Reported hardness and alkalinity of Lester water, 100 and 97 mg/l $CaCO_3$ respectively,
- Reported pH of Lester water is 8.0-8.5,
- DOC concentration of 5.7 mg/l was estimated from the reported TOC concentration in the Lester water (i.e. 7.1 mg/l),
- Concentration series (6 concentrations between 9.9 and 237 µg/l Cu for *C. dubia)* and dose-response curve are reported,

- Dose response curve for reproduction and mortality for *C. dubia* are reported,
- Control mortality for *C. dubia* is 10%,
- Statistics are reported,
- Reliable NOEC values for *C. dubia* are **122** (mortality) and **31.6** (reproduction) µg/l Cu,

9. Belanger *et al.*, 1989

Comments:

- Background Cu concentrations in control water are reported, 1.5-3.9 µg/l Cu for New River water/ Clinch river water (2.9-6.3 µg/l Cu),
- River water was filtred over 11 µm,
- Culture water is the New River/ Clinch river water,
- Reported hardness and alkalinity of New River are 94 and 69.6 mg/l $CaCO_3$ respectively,
- Reported hardness and alkalinity of Clinch River are 179 and 140 mg/l $CaCO_3$ respectively,
- Reported for New River and Clinch River pH is 8.15 and 8.31 respectively,
- DOC concentration of respectively 3.0/2.9 mg/l was estimated from the reported TOC concentration in the Clinch and New River water (i.e. 3.7/3.65 mg/l; source: USGS database),
- Cu concentrations reported in the food (algae : between 35.7 and 73.2 µg/g dw),
- Concentration series are reported (6 concentrations between 6.3 and 33.8 µg/l Cu ; 2 concentrations 10.5 and 21.9 µg/l Cu) for New River,
- Concentration series are reported (6 concentrations between 19.3 and 122.5 µg/l Cu ; 2 concentrations 24.1 and 52.3 µg/l Cu) for Clinch River,
- Statistics are reported,
- No control mortality,
- Dose-response curve is reported; reliable NOEC value for *C. dubia* (reproduction) for Clinch river experiments is **24.1** µg/l Cu,
- Dose-response curve is reported; reliable NOEC value *C. dubia* (reproduction) value for New river experiments is **6.3** µg/l Cu.

10. Van Leeuwen *et al.*, 1988

Comments:

- Background Cu concentrations in control water (filtred Lake Ijssel water) are reported (2.6 µg/l Cu)
- Nominal concentration never deviated more than 10% from nominal concentration,
- Mean alkalinity of testmedia is not reported,
- Hardness of testmedia is 225 mg/l $CaCO_3$,
- Reported pH is 8.1,
- DOC concentration was estimated from the values reported Santore et al. (2002), i.e. 2.0 mg/l for natural lake waters,
- Statistics are reported,
- Dose reponse curve for mortality/ growth is clear,
- No dose response curve for rate of increase,
- Mortalities in control is 4%,
- 5 Cu concentraton (110-3.9 µg/l Cu),
- NOEC calculation for *D. magna* on rate of increase not possible because in 110 µg/l Cu concentration all organisms died !,
- Reliable NOEC values for *D. magna* should therefore be **36.8** (mortality), **36.8** (population growth) and **12.6** µg/l Cu (growth).

11. Heijerick et al., 2002

Comments:

- All tests were performed according to the OECD guidelines (N°202 for *Daphnia magna*),
- Background Cu concentrations in control water (reconstituted) are not reported,
- Mean hardness of testmedia varied between 7.9 and 238 mg/l $CaCO_3$ for daphnid tests,
- Natural DOC extracted from rivers and lakes (between 1.99 and 20.4 mg/l),
- Reported pH value varied between 6.14 and 8.3 for the daphnid tests,
- Statistics are reported,
- Dose response curve are reported,
- Cu concentration tested (5 concentrations and 1 control),
- Reliable NOEC (21 days of exposure) values for *Daphnia magna* are (endpoint reproduction): **28, 21.5, 71.4, 68.8, 106, 181** µg/l Cu.

12. Winner, 1985

Comments:

- Background Cu concentrations in control water (ultrapure reconsituted water from distilled, carbon filtred, deionised water) are not reported,
- Testwater contains organics at concentrations which are below detection limit,
- Measured concentrations never deviate more than 10% from the nominal values,
- DOC added as Aldrich humic acids at 0.15, 0.75 and 1.5 mg/l to ultrapure water containing 0.1 mg/l DOC (according to Santore et al., 2002) resulting in final estimated DOC concentrations of 0.1 (no DOC addition), 0.18, 0.48 and 0.85 mg/l,
- Mean reported hardness and alkalinity of testmedia are 58-115-230 and 115 mg/l $CaCO_3$,
- Reported pH ranges from 8.4 to 8.7,
- Cu concentration series are reported (concentration series vary between treatments ; minimum 3 Cu concentrations tested and 1 control),
- Dose-response curve mortality is reported,
- Control survival between 80 and 100%,
- Renewal of test water every 2 to 3 days,
- Statistics are reported,
- The following reliable NOEC values for *D. pulex* on survival are derived:
  - soft water **: 4, 20** and **30** µg/l,
  - medium hard water : **5, 20** and **40** µg/l,
  - hard water : **10, 15** and **20** µg/l.

13. De Schamphelaere et al., 2006

Comments:

- Background Cu concentrations (reconstituted water with added natural DOC from the Ankeveensche Plassen) in control water is 0.3 µg/l,
- Tests were performed in static systems,
- Reported hardness of testmedia is 100 mg/l $CaCO_3$,
- Tests were performed at pH values of 6 and 7.8; and at DOC concentrations of 5 and 15 mg/l,
- Individual Cu concentration series are reported (5 test concentrations between 7.5 and 270.2 µg/l depending on the experiment),
- Exposure time: different neonates (<2 h) were exposed for 48 h (= full life cycle) at 25°C,
- Dose-response curve is reported,
- Intrinsic rates of increase is reported as endpoint,
- Copper concentrations were determined using a flame-AAS or a graphite furnace AAS,
- Statistics are reported,
- Reliable NOEC values for the rotifer *B. calyciflorus* are **8.2**, **31.2**, **47.8** and **103** µg Cu/l depending on the pH and DOC of the test media.

14. Maund *et al.*, 1992

Comments:

- Background Cu concentrations in control water (dechlorinated tapwater that passed through a copper selective chelating resin) are reported: 2.6 µg/l,
- Measured and nominal concentration never deviated more than 10%,
- Origin organisms: river Ely in South Wales,
- Mean hardness of testmedia is 103 mg/l $CaCO_3$,
- Mean reported pH value is 7.9,
- DOC concentration was estimated from the values reported Santore et al. (2002), i.e. 1.0 mg/l for tap waters,
- Statistics are reported,
- 4 Cu concentration tested (11.0-23.1 µg/l Cu),
- Dose response was observed,
- Reliable NOEC data for *Gammarus pulex* (mean population density) is **11.0** µg/l Cu.

15. Deaver and Rodgers, 1996

Comments:

- Control water: UMBFS spring water,
- Mean copper recovery was 91.8%,
- Background Cu concentrations in control water are not reported,
- 6 Cu concentrations tested: only shown in graph,

- Mean alkalinity and hardness of testmedia are reported 10-63 and 10-64 mg/l $CaCO_3$,
- pH value reported ranges from 6.9 to 8.0,
- DOC concentration was estimated from the values reported Santore et al. (2002), i.e. 1.0 mg/l for spring waters,
- Statistics reported,
- Dose reponse curve for mortality are reported,
- Control mortality are < 10%,
- Reliable NOEC values for *H. azteca* are **30, 50, 50, 82** and **82** (mortality) µg/l Cu.

16. Othman and Pascoe, 2002

Comments:

- Background Cu concentrations (dechlorinated tapwater) in control water is 3.0 µg/l,
- Tests were performed in static renewal systems,
- Reported hardness of testmedia is 128 mg/l $CaCO_3$,
- Tests were performed at a pH value of 7.6,
- Individual Cu concentration series are reported (4 test concentrations between 13.0 and 212.5 µg/l; 3 replicates),
- DOC concentration was estimated as 1.0 mg/l for tapwaters (Santore et al., 2002),
- Equilibration time of 72 h,
- Exposure time: different neonates (<7 days old) were exposed for 35 days at 22°C,
- Dose-response curve is reported,
- Mortality is reported as endpoint,
- Copper concentrations were determined using a ICP MS,
- Statistics are reported (Anova and Tukey-Kramer comparison),
- Reliable NOEC values for the rotifer *H. azetca* is **32** µg Cu/l.

17. Taylor *et al.,* 1991

Comments:

- Background Cu concentrations in the reconstitued control waters are not reported,
- Culture water & organisms: no information,
- Mean hardness of testmedia is 151 mg/l,
- Mean reported pH value is 7.0,
- DOC concentration was estimated as 0.5 mg/l for reconstituted waters
- Statistics are reported,
- 5 Cu concentration tested, 8.8-50 µg/l Cu,
- Dose response was found,
- Reliable NOEC data for *Chironomus riparius* is **16.9** µg/l (growth).

18. Nebeker *et al.*, 1984

Comments:

- Background Cu concentrations in control water/ culture water (Western Fish Toxicology Station in Oregon) are not reported,
- Well water is used as test water,
- Reported hardness and alkalinity of testmedia are 26 and 26 mg/l $CaCO_3$ respectively,
- Reported pH values is varying between 7.2 and 7.4,
- DOC concentration was estimated from the values reported Santore et al. (2002), i.e. 1.3 mg/l for well waters,
- Cu concentration series are reported (10 concentrations between 4.2 and 98 µg/l Cu),
- A clear dose-response curve is reported,
- Control mortality is 20%,
- Statistics are reported,
- Reliable NOEC values for *C. magnifica* are **8.3** and **13** (life cycle) µg/l Cu.

19. Hatakeyama and Yasuno, 1981

Comments:

- Control water: reconstituted artificial soft water; salts were added in distilled water,
- Background Cu concentrations in control water are not reported,
- 9 Cu concentrations tested (2560-10 µg/l Cu),
- No information concerning the culture water ?
- Mean alkalinity is not reported and hardness of testmedia is 25 mg/l $CaCO_3$,

- Reported pH is 6.9,
- DOC concentration for reconstituted waters was estimated as i.e. 0.5 mg/l,
- Statistics are used but methodology is not reported,
- Endpoint of tests is wing length of emerged adults,
- Dose reponse curve for *P. parthenogeneticus* (reproduction/ growth - wing length) is not clear ; Reliable NOEC value at **40** (growth) and **40** µg/l Cu (reproduction), if statistics are properly applied.

20. Kraak et al., 1994

Comments:

- Background Cu concentrations (Lake Markermeer in The Netherlands) in control water is 2.0 µg/l,
- Tests were performed in static renewal systems,
- Reported hardness of testmedia is 150 mg/l $CaCO_3$,
- Tests were performed at a pH value of 7.9,
- DOC level of Markermeer (origin of the test water) was used as a basis for the DOC estimation; the Markermeer water was however filtered extensively over a sand bed to reduce the TOC (pers. communication) and the resulting DOC value is therefore < 7.3 mg/L.
- Individual Cu concentration series are reported (8 test concentrations; 2 replicates),
- Exposure time: 1.6 to 2.0 cm mussels were exposed for 2 days at 15°C,
- Clear dose-response curve is reported,
- Filtration rate is reported as endpoint,
- Copper concentrations were determined using AAS,
- Statistics are reported (Anova and Scheffe's comparison),
- Reliable NOEC values for the mussel *Dreissenia polymorpha* is **13** µg Cu/l.

21. Mersch et al., 1994

Comments:

- Background Cu concentrations (tapwater) in control water is 4.5 µg/l,
- Tests were performed in flow through systems,
- Reported hardness of testmedia is 296 mg/l $CaCO_3$,
- Tests were performed at a pH value of 7.8,
- DOC concentration was estimated as 1.0 mg/l for tapwaters (Santore et al., 2002),
- Individual Cu concentration series are reported (3 test concentrations),
- Exposure time: 18 to 22 mm mussels were exposed for 27 days at 14°C,
- Clear dose-response curve is reported,
- Filtration rate is reported as endpoint,
- Copper concentrations were determined using AAS,
- Statistics are reported (Paired Student t-test)),
- Reliable NOEC values for the mussel *Dreissenia polymorpha* is **21** µg Cu/l.

22. Jacobson *et al.*, 1997

Comments:

- Background Cu concentrations in control water (Clinch river water) are reported (3.2 µg/l Cu),
- Mean alkalinity and hardness of testmedia is 132 and 152 mg/l $CaCO_3$,
- Reported pH value is 8.39,
- DOC concentration of respectively 3.0 mg/l was estimated from the reported TOC concentration in the Clinch River water (i.e. 3.7 mg/l; source: USGS database),
- Statistics are reported,
- Control survival is 97%,
- 2 Cu concentration tested (10.6 and 19.1 µg/l Cu),
- No dose response was observed,
- Reliable NOEC for survival for *Villosa iris* was **19.1**µg/l Cu.

23. Arthur and Leonard, 1970

Comments:

- Background Cu concentrations in control water (= tapwater originated from Lake Superior) are reported 1.9-2 µg/l,
- Acclimation time to the testwater between 10 days and 5 weeks,
- Origin of the organisms: St Croix and eau Claire rivers in the vicinity of Gordon,
- Reported hardness and alkalinity of testmedia are 35-55 and 42.7 mg/l $CaCO_3$ respectively,
- Reported pH value ranges between 7.1 and 8.15,

- DOC concentration was estimated from the values reported Santore et al. (2002), i.e. 1.0 mg/l for tapwaters,
- Cu concentration series are reported (5 concentrations between 28 and 2.9 µg/l Cu and 1 control of 1.9-2 µg/l Cu),
- Dose-response curve for mortality for both species is reported, but no statistics are reported (no significance testing). By own statistical analysis ($p<0.05$) the following NOEC data could be calculated: NOEC for *G. pseudolimnaeus* (mortality): 6.2, 8 and 8 µg/l; NOEC for *P. integra* (mortality): 8 and 14.8 µg/l; NOEC for *C. decisum* (mortality): **8** and **8** µg/l,
- Control mortality for *G. pseudolimnaeus* between 30 and 45%,
- Control mortality for *C. decisum* between 5 and 15%,
- Control mortality for *P. integra* between 40 and 55%.
- Rjected mortality data for *G. pseudolimnaeus* and *P. integra*: high control mortalities (>20%), i.e. between 30 and 75%.

24. Nebeker *et al.*, 1986

Comments:

- Background Cu concentrations in control water/ culture water (origin: coastal stream Oregon) are not reported,
- Well water is used as test water,
- Reported hardness and alkalinity of testmedia are 21 and 28 mg/l $CaCO_3$ respectively,
- Reported pH values is 7.1,
- DOC concentration was estimated from the values reported Santore et al. (2002), i.e. 1.3 mg/l for well waters,
- Individual Cu concentration series are not reported (between 5 and 10; dilution rate of 0.7),
- Dose-response curve is not reported,
- Control mortality is not reported,
- Statistics are reported,
- Reliable NOEC values for *Juga plicifera* is **6** (mortality) µg/l Cu.

Table 2.3: Summary of the NOEC values and physico-chemical parameters for freshwater fish. Selected high quality Q1 NOEC values are underlined selected for the effects assessment and bioavailability normalisation

Organism	Age/size of organisms	Test substance (& purity)	Exposure time	Endpoint	NOEC (µg/l)	Dose-response	Testtype	Cb (µg Cu/l)	Physico-chemical conditions	Medium	Reference
Ictalurus punctatus	fry	$CuSO_4$ (analytical grade)	60 d	growth	**13**	yes	FT	3	T: 22°C; pH: 7.65; H: 186.3 mg/l CaCO3; DOC: 1.3 mg/l (9)	Well	Sauter et al., 1976 (25)
Ictalurus punctatus	fry	$CuSO_4$ (analytical grade)	60 d	mortality	**13**	yes	FT	3	T: 22°C; pH: 7.65; H: 186.3 mg/l CaCO3; DOC: 1.3 mg/l (9)	Well	Sauter et al., 1976 (25)
Noemacheilus barbatulus	adult (8.7 - 12.1 cm)	$CuSO_4$ (not reported)	64 d	mortality	**120**	yes	FT	2	T 11.9°C; pH: 8.26; H: 249 mg/l CaCO3; DOC: 1.3 mg/l (9)	Well	Solbe & Cooper, 1976 (26)
Oncorhynchus kisutch	parr	Not reported (not reported)	61 d	growth	**22**	/	FT	/	T: 9.5 °C; pH: 7.15; H: 24.4 mg/l CaCO3; DOC: 2.9 mg/l (11)	River (Chehalis River)	Mudge et al., 1993 (27)
Oncorhynchus kisutch	fry	Not reported (not reported)	60 d	growth	**21**	/	FT	/	T: 16.7 °C; pH: 7.4; H: 31.8 mg/l CaCO3; DOC: 2.9 mg/l (11)	River (Chehalis River)	Mudge et al., 1993 (27)
Oncorhynchus kisutch	parr	Not reported (not reported)	61 d	growth	**28**	/	FT	/	T: 8.7 °C; pH: 7.0; H: 28.7 mg/l CaCO3; DOC: 2.9 mg/l (11)	River (Chehalis River)	Mudge et al., 1993 (27)
Oncorhynchus kisutch	parr	Not reported (not reported)	61 d	mortality	**24**	/	FT	/	T: 9.5 °C; pH: 7.15; H: 24.4 mg/l CaCO3; DOC: 2.9 mg/l (11)	River (Chehalis River)	Mudge et al., 1993 (27)
Oncorhynchus kisutch	fry	Not reported (not reported)	60 d	mortality	**18**	/	FT	/	T: 16.7 °C; pH: 7.4; H: 31.8 mg/l CaCO3; DOC: 2.9 mg/l (11)	River (Chehalis River)	Mudge et al., 1993 (27)

Organism	Age/size of organisms	Test substance (& purity)	Exposure time	Endpoint	NOEC (µg/l)	Dose-response	Testtype	Cb (µg Cu/l)	Physico-chemical conditions	Medium	Reference
Oncorhynchus mykiss	fry (0.12 g; 2.6 cm)	$CuCl_2$ (reagent grade)	60 d	growth	**2.2**	yes	FT	0.45*	T: 9.8 °C; pH: 7.5; H: 24.6 mg/l CaCO3; DOC: 0.2 mg/l (12)	Well + deionised water	Marr et al., 1996 (28)
Oncorhynchus mykiss	parr	Not reported (not reported)	61 d	growth	**45**	yes	FT	/	T: 9.5 °C; pH: 7.2; H: 24.4 mg/l CaCO3; DOC: 2.9 mg/l (11)	River (Chehalis River)	Mudge et al., 1993 (27)
Oncorhynchus mykiss	eggs	$CuCl_2$ (not reported)	63 d	growth	**16**	yes	FT	3	T: 12 °C; pH: 7.65; H: 120 mg/l CaCO3; DOC: 1.3 mg/l (9)	Well	Seim et al., 1984 (29)
Oncorhynchus mykiss	parr	Not reported (not reported)	61 d	mortality	**24**	/	FT	/	T: 9.5 °C; pH: 7.15; H: 24.4 mg/l CaCO3; DOC: 2.9 mg/l (11)	River (Chehalis River)	Mudge et al., 1993 (27)
Oncorhynchus mykiss	parr	Not reported (not reported)	61 d	mortality	**28**	/	FT	/	T: 8.7 °C; pH: 7.0; H: 28.7 mg/l CaCO3; DOC: 2.9 mg/l (11)	River (Chehalis River)	Mudge et al., 1993 (27)
Oncorhynchus mykiss	embryo	$CuSO_4$ (reagent grade)	45 d	Growth	**11.4**	yes	FT	3	T: 10.8 °C; pH: 7.6; H: 45 mg/l CaCO3; DOC: 1.0 mg/l (13)	Lake (Lake Superior)	McKim et al., 1978 (30)
Oncorhynchus mykiss	embryo	$CuSO_4$ (reagent grade)	45 d	mortality	**11.4**	yes	FT	3	T: 10.8 °C; pH: 7.6; H: 45 mg/l CaCO3; DOC: 1.0 mg/l (13)	Lake (Lake Superior)	McKim et al., 1978 (30)
Catostomus commersoni	embryo	$CuSO_4$ (reagent grade)	40 d	Growth	**12.9**	yes	FT	3	T: 14.9 °C; pH: 7.6; H: 45 mg/l CaCO3; DOC: 1.0 mg/l (13)	Lake (Lake Superior)	McKim et al., 1978 (30)
Catostomus commersoni	embryo	$CuSO_4$ (reagent grade)	40 d	mortality	**12.9**	yes	FT	3	T: 14.9 °C; pH: 7.6; H: 45 mg/l CaCO3; DOC: 1.0 mg/l (13)	Lake (Lake Superior)	McKim et al., 1978 (30)

Organism	Age/size of organisms	Test substance (& purity)	Exposure time	Endpoint	NOEC (µg/l)	Dose-response	Testtype	Cb (µg Cu/l)	Physico-chemical conditions	Medium	Reference
Esox lucius	embryo	$CuSO_4$ (reagent grade)	35 d	Growth	**34.9**	yes	FT	3	T: 15.6 °C; pH: 7.6; H: 45 mg/l CaCO3; DOC: 1.0 mg/l (13)	Lake (Lake Superior)	McKim et al., 1978 (30)
Esox lucius	embryo	$CuSO_4$ (reagent grade)	35 d	mortality	**34.9**	yes	FT	3	T: 15.6 °C; pH: 7.6; H: 45 mg/l CaCO3; DOC: 1.0 mg/l (13)	Lake (Lake Superior)	McKim et al., 1978 (30)
Perca fluviatilis	juvenile (3.8 - 4.3 g)	$CuSO_4$ (pro analysis)	30 d	growth	**39**	yes	FT	1	T: 17.5 °C; pH: 7.8; H: 194 mg/l CaCO3; DOC: 1 mg/l (7)	Tap	Collvin, 1985 (31)
Perca fluviatilis	juvenile (3.8 g)	$CuSO_4$ (pro analysis)	30 d	mortality	**188**	yes	FT	3	T: 15.1 °C; pH: 7.8; H: 178 mg/l CaCO3; DOC: 1mg/l (7)	Tap	Collvin, 1984 (32)
Pimephales notatus	fry (15 - 16 mm) -second generation	$CuSO_4$ (reagent grade)	30 d	growth	**44**	yes	FT	4.3	T: 25 °C; pH: 8.1; H: 201 mg/l CaCO3; DOC: 0.55 mg/l (14)	Spring + demineralised tap	Horning & Neiheisel, 1979 (33)
Pimephales notatus	fry (15 - 16 mm)	$CuSO_4$ (reagent grade)	60 d	growth	**71.8**	yes	FT	4.3	T: 25 °C; pH: 8.1; H: 201 mg/l CaCO3; DOC: 0.55 mg/l (14)	Spring + demineralised tap	Horning & Neiheisel, 1979 (33)
Pimephales notatus	fry (15 - 16 mm)	$CuSO_4$ (reagent grade)	60 d	mortality	**71.8**	yes	FT	4.3	T: 25 °C; pH: 8.1; H: 201 mg/l CaCO3; DOC: 0.55 mg/l (14)	Spring + demineralised tap	Horning & Neiheisel, 1979 (33)
Pimephales promelas	fry (10 - 15 mm)	$CuSO_4$ (reagent grade)	330 d	growth	**33**	yes	FT	3.5	T: 21°C; pH: 8.0; H: 198 mg/l CaCO3; DOC: 0.55 mg/l (14)	Spring+ deionised tap	Mount, 1968 (34)
Pimephales promelas	fry (10 - 20 mm)	$CuSO_4$ (reagent grade)	327 d	growth	**10.6**	yes	FT	4.4	T: 22°C; pH: 6.9; H: 31.4 mg/l CaCO3; DOC: 0.55 mg/l (14)	Spring+ deionised tap	Mount & Stephan, 1969 (35)

Organism	Age/size of organisms	Test substance (& purity)	Exposure time	Endpoint	NOEC (µg/l)	Dose-response	Testtype	Cb (µg Cu/l)	Physico-chemical conditions	Medium	Reference
Pimephales promelas	larvae (4 weeks old)	$CuSO_4$ (reagent grade)	187 d	growth	**59.5**	yes	FT	4.2	T: 23°C; pH: 7.85; H: 202 mg/l CaCO3; DOC: 0.55 mg/l (14)	Spring+ demineralised tap	Pickering et al., 1977 (36)
Pimephales promelas	embryo-larval	$CuSO_4$ (reagent grade)	32 d	growth	**4.8**	yes	FT	1.25*	T: 25°C; pH: 7.05; H: 44 mg/l CaCO3; DOC: 1 mg/l (13)	Lake (Lake Superior)	Spehar & Fiandt, 1985 (37)
Pimephales promelas	fry (10 - 15 mm)	$CuSO_4$ (reagent grade)	330 d	mortality	**33**	yes	FT	3.5	T: 21°C; pH: 8.0; H: 198 mg/l CaCO3; DOC: 0.55 mg/l (14)	Spring+ deionised tap	Mount, 1968 (34)
Pimephales promelas	fry (10 - 20 mm)	$CuSO_4$ (reagent grade)	327 d	mortality	**10.6**	yes	FT	4.4	T: 22°C; pH: 6.9; H: 31.4 mg/l CaCO3; DOC: 0.55 mg/l (14)	Spring+ deionised tap	Mount & Stephan, 1969 (35)
Pimephales promelas	larvae	$CuSO_4$ (not reported)	28 d	mortality	**61**	yes	FT	0.6	T: 21°C; pH: 8.17; H: 202 mg/l CaCO3; DOC: 1.3 mg/l (9)	Ground water	Scudder et al., 1988 (38)
Pimephales promelas	embryo-larval	$Cu(NO_3)_2$ (reagent grade)	32 d	mortality	**4.8**	yes	FT	1.25*	T: 25°C; pH: 7.05; H: 44 mg/l CaCO3; DOC: 1 mg/l (13)	Lake (Lake Superior)	Spehar & Fiandt, 1985 (37)
Pimephales promelas	juvenile (32 - 38 mm; 5 months old)	$CuSO_4$ (reagent grade)	270 d	reproduction	**66**	yes	FT	7	T: 23°C; pH: 8.1; H: 274 mg/l CaCO3; DOC: 2 mg/l (3)	River	Brungs et al., 1976 (39)
Pimephales promelas	fry (10 - 15 mm)	$CuSO_4$ (reagent grade)	330 d	reproduction	**14.5**	yes	FT	3.5	T: 21°C; pH: 8.0; H: 198 mg/l CaCO3; DOC: 0.55 mg/l (14)	Spring+ deionised tap	Mount, 1968 (34)
Pimephales promelas	fry (10 - 20 mm)	$CuSO_4$ (reagent grade)	327 d	reproduction	**10.6**	yes	FT	4.4	T: 22°C; pH: 6.9; H: 31.4 mg/l CaCO3; DOC: 0.55 mg/l (14)	Spring+ deionised tap	Mount & Stephan, 1969 (35)

Organism	Age/size of organisms	Test substance (& purity)	Exposure time	Endpoint	NOEC (µg/l)	Dose-response	Testtype	Cb (µg Cu/l)	Physico-chemical conditions	Medium	Reference
Pimephales promelas	larvae (4 weeks old)	$CuSO_4$ (reagent grade)	187 d	reproduction	**25.5**	yes	FT	4.2	T: 23°C; pH: 7.9; H: 202 mg/l CaCO3; DOC: 0.55 mg/l (14)	Spring+ deionised tap	Pickering et al., 1977 (36)
Pimephales promelas	larvae (4 weeks old)	$CuSO_4$ (reagent grade)	97 d	reproduction	**23**	yes	FT	4.2	T: 23°C; pH: 7.9; H: 202 mg/l CaCO3; DOC: 0.55 mg/l (14)	Spring+ deionised tap	Pickering et al., 1977 (36)
Pimephales promelas	larvae (4 weeks old)	$CuSO_4$ (reagent grade)	7 d	reproduction	**22.5**	yes	FT	4.2	T: 23°C; pH: 7.9; H: 202 mg/l CaCO3; DOC: 0.55 mg/l (14)	Spring+ deionised tap	Pickering et al., 1977 (36)
Salvelinus fontinalis	embryo	$CuSO_4$ (reagent grade)	60 d	Growth	**22.3**	yes	FT	/	T: 5.6 °C; pH: 7.6; H: 45 mg/l CaCO3; DOC: 1.0 mg/l (13)	Lake (Lake Superior)	McKim et al., 1978 (30)
Salvelinus fontinalis	embryo	$CuSO_4$ (reagent grade)	60 d	mortality	**22.3**	yes	FT	/	T: 5.6 °C; pH: 7.6; H: 45 mg/l CaCO3; DOC: 1.0 mg/l (13)	Lake (Lake Superior)	McKim et al., 1978 (30)
Salvelinus fontinalis	Alevins/juveniles	$CuSO_4$ (reagent grade)	189 d	Growth	**9.5**	yes	FT	/	T: 10.6 °C; pH: 7.5; H: 45 mg/l CaCO3; DOC: 1 mg/l (7)	Tap	McKim & Benoit, 1971 (40)
Salvelinus fontinalis	Alevins/juveniles	$CuSO_4$ (reagent grade)	189 d	mortality	**9.5**	yes	FT	/	T: 10.6 °C; pH: 7.5; H: 45 mg/l CaCO3; DOC: 1 mg/l (7)	Tap	McKim & Benoit, 1971 (40)
Salvelinus fontinalis	yearling	$CuSO_4$ (reagent grade)	244 d	growth	**17.4**	yes	FT	/	T: 10.6 °C; pH: 7.5; H: 45 mg/l CaCO3; DOC: 1 mg/l (7)	Tap	McKim & Benoit, 1971 (40)
Salvelinus fontinalis	fry	$CuSO_4$ (analytical grade)	30 d	Growth	**7**	yes	FT	3	T: 10 °C; pH: 6.85; H: 37.5 mg/l CaCO3; DOC: 1.3 mg/l (9)	Well	Sauter et al., 1976 (25)

Organism	Age/size of organisms	Test substance (& purity)	Exposure time	Endpoint	NOEC (µg/l)	Dose-response	Testtype	Cb (µg Cu/l)	Physico-chemical conditions	Medium	Reference
Salvelinus fontinalis	fry	$CuSO_4$ (analytical grade)	30 d	growth	**21**	yes	FT	3	T: 10 °C; pH:6.9; H: 187 mg/l CaCO3; DOC: 1.3 mg/l (9)	Well	Sauter et al., 1976 (25)
Salvelinus fontinalis	yearling	$CuSO_4$ (reagent grade)	244 d	mortality	**17.4**	yes	S	1.9	T: 10.6 °C; pH: 7.45; H: 45 mg/l CaCO3; DOC: 1 mg/l (7)	Tap	McKim & Benoit, 1971 (40)
Salvelinus fontinalis	fry	$CuSO_4$ (analytical grade)	60 d	mortality	**13**	yes	FT	3	T: 10 °C; pH: 6.85; H: 37.5 mg/l CaCO3; DOC: 1.3 mg/l (9)	Well	Sauter et al., 1976 (25)
Salvelinus fontinalis	fry	$CuSO_4$ (analytical grade)	30 d	mortality	**21**	yes	FT	3	T: 10 °C; pH:6.9; H: 187 mg/l CaCO3; DOC: 1.3 mg/l (9)	Well	Sauter et al., 1976 (25)
Salvelinus fontinalis	yearling	$CuSO_4$ (reagent grade)	244 d	reproduction	**17.4**	yes	FT	1.9	T: 10.6 °C; pH: 7.45; H: 45 mg/l CaCO3; DOC: 1 mg/l (7)	Tap	McKim & Benoit, 1971 (40)
Salvelinus fontinalis	fry	$CuSO_4$ (analytical grade)	60 d	reproduction	**7**	yes	FT	3	T: 10 °C; pH: 6.85; H: 37.5 mg/l CaCO3; DOC: 1.3 mg/l (9)	Well	Sauter et al., 1976 (25)
Salvelinus fontinalis	fry	$CuSO_4$ (analytical grade)	30 d	reproduction	**49**	yes	FT	3	T: 10 °C; pH:6.9; H: 187 mg/l CaCO3; DOC: 1.3 mg/l (9)	Well	Sauter et al., 1976 (25)

- DOC concentrations:
(1): DOC estimation of reconstituted water is 0.5 mg/l (De Schamphelaere and Janssen, 2002 (0.3 mg DOC/L); Ryan eta al., 2004 (0.4-0.5 mg DOC/L); Karman et al., 2004 (<0.1 mg DOC/L); Hollis et al, 1997 (0.4-0.6 mg DOC/L).
(2): DOC estimation for New River (USA) water extracted from the United States Geological Survey records (USGS). The USGS database reports TOC concentration of 3.65 mg/l, and assuming a DOC/TOC ratio of 0.8.
(3): DOC estimation for unknown river/lake water or for which no reliable DOC concentration could be estimated is 2.0 mg/l (Santore et al., 2002)
(4): DOC estimation for Clinch River (USA) water extracted from the United States Geological Survey records (USGS). The USGS database reports TOC concentration of 3.7 mg/l, and assuming a DOC/TOC ratio of 0.8.
(5): DOC estimation for Lester River (USA) water extracted from the United States Geological Survey records (USGS). The USGS database reports TOC concentration of 7.1 mg/l, and assuming a DOC/TOC ratio of 0.8.

(6): DOC estimation for deionized water (= 0.1 mg/l according to Santore et al., 2002) with addition of artificial humic acids (no addition; 0.15 mg/l; 0.75 mg/l; 1.5 mg/l). Conversion from humic acid content to organic carbon content was performed after using a factor of 2.
(7): DOC estimation for tap water is 1.0 mg/l (Santore et al., 2002)
(8): DOC estimation for spring water is 1.0 mg/l (Santore et al., 2002)
(9): DOC estimation for well water is 1.3 mg/l (Santore et al., 2002)
(10): DOC level of Markermeer (origin of the test water) was used as a basis for the DOC estimation; the Markermeer water was however filtered extensively over a sand bed to reduce the TOC (pers. communication)) and the resulting DOC value is therefore < 7.3 mg/L.
(11): DOC estimation for Chehalis River (USA) water extracted from the United States Geological Survey records (USGS). The USGS database reports TOC concentration of 3.6 mg/l, and assuming a DOC/TOC ratio of 0.8.
(12): DOC estimation for ultrapure deionized water (0.1 mg/l Santore et al., 2002) and well water (1.3 mg/l according to Santore et al., 2002) in a ratio of 90%/10% is 0.45 mg/l.
(13): DOC estimation for Lake Superior water is 1.0 mg/l (Santore et al., 2002)
(14): DOC estimation for demineralised/deionized water (0.1 mg/l Santore et al., 2002) and spring water (1.0 mg/l according to Santore et al., 2002) in a ratio of 50%/50% is 0.55 mg/l.
- test type
S: static; R: renewal; FT: flow through

25. Sauter *et al.*, 1976 (EPA document)

Comments:

- Background Cu concentrations in control water (Bedrock well) is 3 µg/l Cu,
- No information was provided on acclimation conditions/background concentrations,
- Mean alkalinity and hardness of testmedia is 27.8-177.6 and 35-170 mg/l $CaCO_3$,
- pH value 6.6 to 7.8,
- DOC concentration was estimated from the values reported Santore et al. (2002), i.e. 1.3 mg/l for well waters,
- Statistics are reported,
- Dose reponse curve for mortality/ growth is clear,
- Mortalities in control for *Salvelinus fontinalis* is 2-4% (soft water) and 0-42% (hard water) after 60 days of exposure (test not acceptable),
- Incubation time for *Salvelinus fontinalis* is 35 days,
- Reliable NOEC for *Salvelinus fontinalis* should be **13** (survival) for 60 days of exposure and **7** µg/l Cu (growth) for 30 days of exposure in soft water; **21** (survival) and **21** µg/l Cu (growth) after 30 days of exposure in hard waters,
- Other reliable NOEC for *Salvelinus fontinalis* are **7** µg/l Cu (reproduction) and **49** µg/l Cu (reproduction) in soft water and hard waters respectively,
- 5 Cu concentrations (95-5 µg/l Cu) for *Salvelinus fontinalis*,
- Mortalities in control for *Ictalurus punctatus* is 24-34% (soft water ; test not acceptable) and 0% (hard water) after 60 days of exposure,
- Incubation time for *Ictalurus punctatus* is 6-8 days,
- NOEC for *Ictalurus punctatus* for soft water testing could be calculated for 30/60 days of exposure, i.e. 12 and 12 µg/l but high control mortalities; NOEC of **13** (survival) and **13** µg/l Cu (growth) after 60 days of exposure in hard waters
- 5 Cu concentrations (3-66 µg/l Cu) for *Ictalurus punctatus*,
- Mortalities in control for *Stizostedion vitreum* is 82% (test not acceptable) and 46-39% (test not acceptable) after 30 days of exposure,
- NOEC for *Stizostedion vitreum* for soft and hard water testing could not be calculated for 30 days of exposure but high control mortalities,
- 5 Cu concentrations for *Stizostedion vitreum* (3-92 µg/l).
- Rejected data for *S vitreum:* high control mortality, i.e. > 39%,
- Rejected data for *I. punctatus* in soft water: high control mortality, i.e. > 24%.

26. Solbe and Cooper, 1976

Comments:

- Background Cu concentrations in control water are reported (2 µg/l Cu),
- Culture water ? (origin Staffordshire stream; 15 months of acclimation),
- Mean hardness of testmedia is 249 mg/l $CaCO_3$,
- Reported pH value is 8.26,
- DOC concentration was estimated from the values reported Santore et al. (2002), i.e. 1.3 mg/l for well waters waters,
- Statistics for NOEC calculation are reported,
- Dose response curve not reported,
- No control mortality,
- 6 Cu concentrations (120-760 µg/l Cu) and 1 control,
- Reliable NOEC data for *N. barbalutus* (survival) is **120** µg/l Cu.

27. Mudge *et al.*, 1993

Comments:

- Background Cu concentrations in control/ culture water (organisms from hatchery followed by 2 weeks of acclimation in Chehalis river water, Washington) are not reported,
- Mean reported hardness and alkalinity of testmedia are 24-32 and 14.8-32.4 mg/l $CaCO_3$,
- Reported pH ranges from 6.6 to 7.9,
- DOC concentration of 2.9 mg/l was estimated from the reported TOC concentration in the Chehalis River water (i.e. 3.6 mg/l; source: USGS database),
- Cu concentration series are not reported (5 concentrations and 1 control of ? µg/l Cu),
- Dose-response curve mortality/growth is not reported (only NOEC values),
- Statistics are used,
- No control mortalities reported,

- Reliable NOEC values for *O. mykiss* are : (1) for mortality **24** and **28** µg/l Cu ; (2) for growth **45** and >51 µg/l Cu,
- Reliable NOEC values for *O. kisuth* are : (1) for mortality **18, 24** and >51µg/l Cu ; (2) for growth **21, 22** and **28** µg/l Cu.

28. Marr et al., 1996

Comments:

- Background Cu concentrations in control water (well water treated with filtration, reverse osmosis and deionization mixed with well water in a ratio of 90%/10%) are reported (<0.9 µg/l Cu),
- 14 days of acclimation in control water,
- flow-through system with 36 volumes renewal per day,
- Mean alkalinity and hardness of testmedia is 25 and 28 mg/l $CaCO_3$,
- Reported pH value is 7.47,
- DOC concentration of 0.2 mg/l was estimated from the values reported Santore et al. (2002), i.e. 1.3 mg/l for well waters and 0.1 mg/l for deionised waters (taking a 90% deionised water and 10% well into account),
- Statistics are reported,
- 4 Cu concentration tested (1.1 – 9 µg/l Cu),
- Dose response curve for growth is reported,
- Cu concentration in feeding 8.7 mg/kg,
- Reliable NOEC data for *O. mykiss* (growth) is **2.2** µg/l Cu.

29. Seim *et al.*, 1984

Comments:

- Control water (well water),
- Background Cu concentrations in control water are reported (3 µg/l),
- 6 Cu concentrations (121-6 µg/l Cu),
- Origin fish: Alsea Fish hatchery, Oregon; acclimation 6 days), ,
- Mean hardness and alkalinity is reported, 120 and 126 mg/l $CaCO_3$,
- pH value ranges between 7.4 and 7.9,
- DOC concentration was estimated from the values reported Santore et al. (2002), i.e. 1.3 mg/l for well waters,
- Statistics are reported,
- Control survival is >90%,
- Dose reponse curve for growth is clear,
- Reliable NOEC value for *O. mykiss* is **16** (growth) µg/l Cu.

30. McKim et al., 1978

Comments:

- Background Cu concentrations (Lake Superior) in control water is 3.0 µg/l,
- Tests were performed in flow-through systems,
- Reported hardness and alkalinity of testmedia is respectively 45.4 mg/l and 42.4 mg/l $CaCO_3$,
- Reported pH values is 7.6,
- DOC concentration was estimated as 1.0 mg/l for Lake Superior watyer (Santore et al., 2002),
- Individual Cu concentration series are reported (6 test concentrations between 4 and 1000 µg/l),
- Exposure time: different embryo stages and 30-60 days after hatching,
- Dose-response curve is reported,
- High control mortality was reported for 2 species i.e. Corogenus artedi and Micropterus sp.,
- Statistics are reported,
- Reliable NOEC values for *O. mykiss* is **11.4** (survival, growth) µg/l Cu; *S. fontanilis* **22.3** (survival, growth) µg/l Cu; *Catostomus commersoni* **12.9** µg/l Cu and *Esox lucius* **34.9** µg/l Cu.
- Rejected data: NOEC values for the fish species Corogonus and Micropterus could not be used because of the high control mortality.

31. Collvin, 1985

Comments:

- Background Cu concentrations in control water (tapwater) are reported (1 µg/l Cu),
- Fish were caught from lake Sovdeborgssjon in Sweden; 4 weeks of acclimation in control water,
- Mean alkalinity and hardness of testmedia is 129 and 194 mg/l $CaCO_3$,
- Reported pH value is 7.8,

- DOC concentration was estimated from the values reported Santore et al. (2002), i.e. 1.0 mg/l for tapwaters,
- Statistics are reported,
- 4 Cu concentration tested (13-81 µg/l Cu) and 1 control,
- Dose response curve is reported,
- Reliable NOEC value for *P. fluviatilis* on growth is 22 µg/l Cu for 18 days and **39** µg/l Cu for 30 days.

32. Collvin, 1984

Comments:

- Background Cu concentrations in control water (tap water) are reported 3 µg/l,
- Acclimation of the organisms in tapwater,
- Origin of the organisms: caught in south swedish lake and kept in tap water with background concentration of 3 µg/l,
- Mean reported hardness and alkalinity of testmedia are 178 and 131 mg/l $CaCO_3$
- Reported pH is 7.8,
- DOC concentration was estimated from the values reported Santore et al. (2002), i.e. 1.0 mg/l for tapwaters
- Cu concentration series are reported (5 concentrations between 700 and 87 µg/l and 1 control of 3 µg/l Cu),
- Dose-response curve mortality is reported, but the statistical treatment is not reported ('all fish died at 492 and 700 µg/l Cu, while all fish exposed to 3, 87, 145 and 188 µg/l Cu survived'),
- No mortalities in control,
- Reliable NOEC value (30 days of exposure) for *P. fluviatilis* is **188** (mortality) µg/l Cu.

33. Horning and Neiheisel, 1979

Comments:

- Background Cu concentrations in control water (spring water from Newton Fish Farm and demineralised tapwater from Cincinnatti) are reported (4.3 µg/l),
- Origin fish: Shayler Run Creek, Ohio, 7 weeks of acclimation in control water,
- Mean reported hardness and alkalinity of testmedia are 172-230 and 150-186 mg/l $CaCO_3$,
- pH ranges between 7.9 and 8.3,
- DOC concentration of 0.55 mg/l was estimated from the values reported Santore et al. (2002), i.e. 1.0 mg/l for spring waters and 0.1 mg/l for deionised waters,
- Cu concentration series are reported (5 concentrations between 18-120 µg/l and 1 control of 4.3 µg/l Cu),
- Dose-response curve mortality/ growth is not reported,
- Dose-response curve reproduction is reported,
- NOEC value for reproduction is <18 µg/l Cu,
- Reliable NOEC values for *P. promelas* on mortality is: **71.8** µg/l Cu,
- Reliable NOEC value for *P. promelas* on growth is: **71.8** µg/l Cu,
- Reliable NOEC for growth after 30 days of exposure is **44.1** µg/l, after 60 days of exposure 71.8 µg/l Cu ??,
- Control survival (93-100%),
- Statistics are reported.

34. Mount, 1968

Comments:

- Background Cu concentrations in control water (mixture of springwater used in the Newtown Fish hatchery + demineralised Cincinnati tapwater) are reported 3.5 µg/l Cu,
- Origin of the organisms: Newtown Fish Farm,
- 6 weeks of acclimation to the testwater,
- Mean reported hardness and alkalinity of testmedia are 198 and 161 mg/l $CaCO_3$,
- Reported pH ranges from 7.5 to 8.5,
- DOC concentration of 0.55 mg/l was estimated from the values reported Santore et al. (2002), i.e. 1.0 mg/l for spring waters and 0.1 mg/l for deionised waters,
- Cu concentration series are reported (4 concentrations between 95 and 5.8 µg/l Cu and 1 control of 4.4 µg/l Cu),
- Dose-response curve for reproduction/growth is reported, but very high variability between replicates,
- 80% survival in control,

- Reliable NOEC (330 days of exposure) for *P. promelas* on mortality, growth and reproduction could be calculated by using own statistical analysis ($p<0.05$), ie **33**, **33** and **14.5** µg/l Cu respectively.

35. Mount and Stephan, 1969

Comments:

- Background Cu concentrations in control water (mixture of springwater used in the Newtown Fish hatchery + demineralised Cincinnati tapwater) are reported 4.4 µg/l Cu,
- Origin of the organisms: Newtown Fish Farm,
- 80% survival in control,
- Mean reported hardness and alkalinity of testmedia are 31 and 30 mg/l $CaCO_3$,
- Reported pH ranges from 6.9 to 7.2,
- DOC concentration of 0.55 mg/l was estimated from the values reported Santore et al. (2002), i.e. 1.0 mg/l for spring waters and 0.1 mg/l for deionised waters,
- Cu concentration series are reported (4 concentrations between 18.4 and 5 µg/l Cu and 1 control of 4.4 µg/l Cu),
- Dose-response curve for reproduction/growth/mortality is reported, but no statistics were applied,
- Reliable NOEC for *P. promelas* on mortality, growth and reproduction could be calculated by using own statistical analysis at $p<0.05$, ie **10.6**, **10.6** and **10.6** µg/l Cu respectively.

36. Pickering *et al.*, 1977

Comments:

- Background Cu concentrations in control water (mixture of springwater used in the Newtown Fish hatchery + demineralised Cincinnati tapwater) are reported (4.2 µg/l),
- All fish were reared from eggs spawned in the laboratory pondwater; larvae were reared for about 4 weeks before they were introduced into the exposure chambers,
- Mean reported hardness and acidity of testmedia are 202 and 8 mg/l $CaCO_3$,
- Reported pH ranges from 7.5 to 8.2,
- DOC concentration of 0.55 mg/l was estimated from the values reported Santore et al. (2002), i.e. 1.0 mg/l for spring waters and 0.1 mg/l for deionised waters,
- Cu concentration series are reported (6 concentrations between 98 and 11 µg/l and 1 control of 4.2 µg/l Cu),
- Dose-response curve mortality is not reported,
- No control mortality,
- Significance testing is used,
- NOEC value for mortality should be >99, >96, >99.5 µg/l Cu,
- Dose response curve for growth/ reproduction is reported,
- Reliable NOEC data for *P. promelas* on reproduction are **22.5, 23** and **25.5** µg/l Cu,
- Reliable NOEC for *P. promelas* on growth could be calculated by using own statistical analysis at $p<0.05$, ie >99.5, >96 and **59.5** µg/l Cu.
- Rejected data for survival: unbounded NOEC.

37. Spehar and Fiandt, 1985 (EPA document)

Comments:

- Background Cu concentrations in control waters are reported, 3.4 µg/l for the Lester water with *C. dubia* and < 2 µg/l for the Lake Superior with *P. promelas.* A background Cu concentration of 1.25 µg/l was retained for Lake Superior according to Poldoski and Glass (1978),
- All the organisms were cultured in their respective water before they were tested,
- Reported hardness and alkalinity of Lester water, 100 and 97 mg/l $CaCO_3$ respectively,
- Reported pH of Lester and Lake Superior water are 8.0-8.5 and 6.0-8.1 respectively,
- Reported hardness and alkalinity of Lake Superior water, 44 and 42 mg/l $CaCO_3$ respectively,
- DOC concentration of 5.7 mg/l was estimated from the reported TOC concentration in the Lester water (i.e. 7.1 mg/l). DOC concentration in Lake Superior water was estimated from Santore et al. (2002), i.e. 1.0 mg/l,
- Concentration series (6 concentrations between 9.9 and 237 µg/l Cu for *C. dubia* and 5 concentrations between 4.8 and 65 µg/l Cu for *P. promelas)* and dose-response curve are reported,
- Dose response curve for growth and mortality for *P. promelas* are reported,
- Control mortality for *P. promelas* is 10%,
- Dose response curve for reproduction and mortality for *C. dubia* are reported,
- Control mortality for *C. dubia* is 10%,
- Statistics are reported,

- Reliable NOEC values for *C. dubia* are **122** (mortality) and **31.6** (reproduction) µg/l Cu,
- Reliable NOEC values for *P. promelas* are **4.8** (mortality) and **4.8** (growth) µg/l Cu,

38. Scudder *et al.*, 1988

Comments:

- Background Cu concentrations in control water (filtred groundwater on Survey property, California) are reported 0.6 µg/l
- A breeding population was established from stocks obtained from the EPA laboratory from Newtown, Ohio,
- Mean reported hardness and alkalinity of testmedia are 202 and 212 mg/l $CaCO_3$,
- Reported pH is 8.17,
- DOC concentration was estimated from the values reported Santore et al. (2002), i.e. 1.3 mg/l for wellwaters,
- Cu concentration series are reported (5 concentrations between 621 and 61 µg/l and 1 control of 0.6 µg/l Cu),
- Dose-response curve mortality/growth is reported,
- Statistics are reported,
- Mortality in control < 20%,
- Reported NOEC values for *P. promelas* on survival are **61** and 338 µg/l Cu, for growth <61 µg/l. NOEC value of 61 µg/l is the only reliable NOEC value.
- Rejected data for growth: unbounded NOEC; and rejected NOEC of 338 µg/l for survival because of both the short term exposure duration (8 days) and the less sensitive life stage (i.e. embryo).

39. Brungs *et al.*, 1976

Comments:

- Background Cu concentrations in control water are reported 7 µg/l Cu,
- 2 weeks of acclimation to the testwater,
- Origin of the organisms: Newton Fish Farm in Ohio,
- Mean reported hardness and alkalinity of testmedia are 274 and 183 mg/l $CaCO_3$,
- Reported pH ranges between 8.0 and 8.3,
- TOC concentration was reported to be 5.9 mg/l,
- Water characteristics vary markedly within the 9 month period: hardness (148-340), alkalinity (76-244), temperature (0-30°C),
- Cu concentration series are reported (6 concentrations between 565 and 33 µg/l Cu and 1 control of 7 µg/l Cu),
- Dose-response curve for mortality is not reported,
- Dose response curve for reproduction (spawning) is reported, but very high variability between replicates and no statistics reported,
- Control mortality is not reported,
- No statistics are used (NOEC for reproduction could be calculated by using own statistical analysis at $p<0.05$),
- Reliable NOEC value for *P. promelas* is **66** (reproduction) µg/l Cu
- Rejected data for mortality : no significance testing used.

40. McKim and Benoit, 1971

Comments:

- Control water (dechlorinated tapwater (Duluth city) taken from Lake Superior),
- Background Cu concentrations in control water are reported (1.9 µg/l),
- Cu concentrations (32.5-3.4 µg/l Cu),
- Origin fish: Grand Lakes Minessota; 3 months of acclimation,
- Mean hardness and alkalinity is reported, 45 and 42 mg/l $CaCO_3$,
- pH value ranges between 6.9 and 8.0,
- DOC concentration was estimated from the values reported Santore et al. (2002), i.e. 1.0 mg/l for tapwaters,
- Statistics are reported,
- Dose reponse curve for mortality/ growth/ reproduction of yearlings is reported,
- Control survival of yearlings is 93%,
- Reliable NOEC for *S. fontanilis* on the considered endpoints should be **17.4**, **17.4** and **17.4** µg/l Cu (mortality/ growth/ reproduction) for yearlings and **9.5, 9.5** µg/l (mortality/ growth) for alevins.

Annexe 3: Acute toxicity and acute-chronic ratios for copper reported in USEPA (2003)

Species	Hardness (mg/L)	Reported Acute Value (µg/L)	Chronic Value (µg/L)	ACR	Species Mean ACR	Reference
C. decisum (snail)	35-55	1673	8.73	191.61	171.19	Arthur and Leonard 1970
C. decisum (snail)	35-55	1673	10.94	152.95		Arthur and Leonard 1970
C. dubia (cladoceran)	94.1	28.42	7.9	3.60	2.90	Belanger et al. 1989
C. dubia (cladoceran)	179	63.33	19.36	3.27		Belanger et al. 1989
C. dubia (cladoceran)	57	13.4	24.5	0.55		Oris et al. 1991
C. dubia (cladoceran)	-	18.974	9.17	2.07		Carlson et al. 1986
D. magna (cladoceran)	51	26	12.58	2.07	3.42	Chapman et al. Manuscript
D. magna (cladoceran)	104	33.76	19.89	1.70		Chapman et al. Manuscript
D. magna (cladoceran)	211	69	6.06	11.39		Chapman et al. Manuscript
D. pulex (cladoceran)	57.5	25.737	2.83	9.10	4.82	Winner 1985
D. pulex (cladoceran)	115	27.6	7.071068	3.90		Winner 1985
D. pulex (cladoceran)	230	28.79	9.16	3.14		Winner 1985
O. mykiss (rainbow trout)	120	80	27.77	2.88	2.88	Seim et al. 1984
O. tshawytscha (chinook salmon)	20-45	33.1	5.92	5.59	5.59	Chapman 1975, 1982
P. notatus (bluntnose minnow)	172-230	231.9	18	12.88	12.88	Horning and Neiheisel 1979
P. promelas (fathead minnow)	45	106.875	9.38	11.40	11.40	Lind et al. 1978
L. macrochirus (bluegill)	21-40	1100	27.15	40.52	40.49	Benoit 1975
C. variegatus (sheepshead minnow)	-	368	249.5276	1.48	1.48	Hughes et al. 1989

Annexe 4: Baseline copper levels and background copper levels

In order to interpret the copper concentrations, it is important to evaluate the data in view of background reference concentrations. “True” natural background concentrations can hardly be found in most European surface waters as a result of historical and current anthropogenic input from diffuse sources. This issue was discussed for the EU Water Framework Directive by a group of experts and the following definition was agreed: “The background concentration of target metals in the aquatic ecosystems of a river basin, river sub-basin or river basin management area is that concentration in the present or past corresponding to very low anthropogenic pressure. The methodologies proposed for setting the background concentrations were: (1) trace metal concentrations in groundwater (shallow and/or deep); (2) analysed values for trace metal concentrations in pristine areas (with assurance that river basin is pristine or nearly so) (3) expert judgment (incl. international agreements; river basin commissions) *(EAF,* 2004). A draft working document discussed further the approach and stated that the first step in this process is to elucidate default background concentrations applicable to a large part of Europe. It was agreed that the most important database is the FOREGS Geochemical Baseline Programme (FGBP) published in March 2004 (http://www.gsf.fi/foregs/geochem/). FOREGS (Forum of European Geological Surveys) Geochemical Baseline Programme sought to provide high quality environmental geochemical baseline data for Europe based on samples of stream water, stream sediment, floodplain sediment, soil, and humus collected all over Europe. High quality and consistency of the obtained data were ensured by using standardised sampling methods and by treating and analysing all samples in the same laboratories. Five random points were selected in each Global Terrestrial Network cell (160*160 km^2), one point in each quadrant and one point random in the cell. The points were used to select the five nearest small drainage basins of <100 km^2. The sampling sites selected for stream water analyses of dissolved metals were typical of locally unimpacted or slightly impacted areas. As a consequence, the metal concentrations – and copper more specific – that are determined in these samples can be considered as relevant background concentrations. These copper concentrations are fundamentally different from the values that were used for the derivation of a RWC-ambient PEC: the surface waters that were used for the RWC-ambient PEC did not represent pristine areas, but only excluded locations that were directly impacted by local point sources.

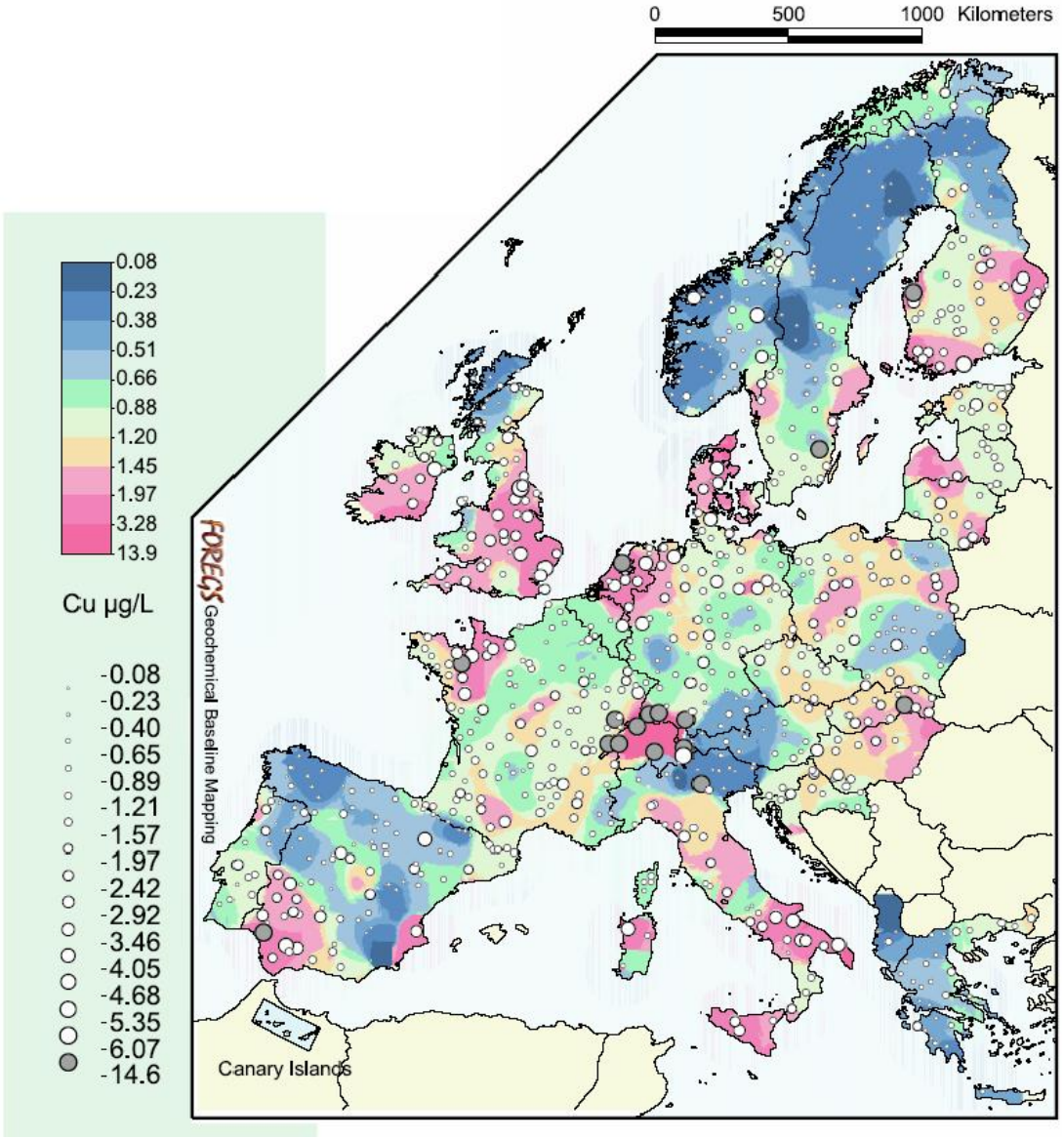
The FOREGS-data set is considered to be of high quality: a detailed description of sampling methodology, sampling preparation and analysis is given by Salminen et al. (2005):

- running stream water was collected form small, second order drainage basins (<100 km^2);
- whenever possible, sampling was performed during winter and early spring months, and was avoided during rainy periods and flood events;
- a full description of sampling materials and sampling volumes is provided, and all materials were rinsed twice with unfiltered or filtered stream water (depending on the type of water sample);
- all potential contaminating factors were reduced during the sampling period (wearing of gloves, no smoking in the area allowed, no hand jewelry was allowed , running vehicles during sampling was prohibited, etc..)

The programme resulted in 807 stream water samples spread over Europe. The interpretation group of FOREGS produced the final stream water maps in their meeting on 3 March 2004. The data that were aquired from the FOREGS monitoring program are shown in Figure 1, which presents the currently most extensive, robust and spatially-relevant data set of dissolved background copper concentrations on the European scale. This map shows the great spatial importance of the copper baseline levels, likely related to local geochemical characteristics. High Cu-values that are found in in Swiss pristine water, for instance, can be related to the physicochemical characteristics of natural

granitic waters that are present in the Alps. Alaux-Negrel et al. (1993) measured elevated concentrations of Ca, Na, Co, W and Zn (Cu not reported) in granitic waters sampled in the Alps.
The total number of analysed (ICP-MS, DEM: 0.005 µg/L) water samples was 807. Dissolved copper ranged between 0.1 and 14 µg Cu/L with 10^{th}/90^{th} percentiles ranging between 0.23 and 3.28 and a 50^{th} percentile of 0.88 µg Cu/L. Taking into account the high quality of the data set, this 50^{th} value is accepted as a typical background concentration for Cu in European surface waters (EU-regional scale).
Background or baseline Cu-concentrations have also been reported in literature. An overview of some relevant background concentrations in EU-waters is given hereunder. For these data a quality assurance is not always possible due to the lack of full, detailed description of all sampling steps.
Study of the metal concentration in lake systems in the Finnish part of Lapland can be considered as a reasonable estimation of natural background concentration for whole Scandinavia (EC, 1998). The metal concentration measured in these aquatic ecosystems were close to the detection limits of the most common used analytical detection methods i.e. FAAS, GFAAS and ICP-AES. The measured total Cu concentration of 0.28 µg/L, calculated as 50^{th} percentile (median), was retained as background concentration for Scandinavian water systems (Mannio et al, 1995). Metal concentrations collected from Finnish catchments (Valkea-Kotinen, Hietajärvi and Pesosjärvi)), located in so called background areas (no point sources of heavy metals), generated similar background concentrations for copper, i.e. between 0.11 and 0.75 µg Cu_{total}/L (reported as 50^{th} percentiles) (Ukonmaanaho et al., 1998). A third study, study covering the whole country (i.e. Finland) with emphasis on the acidified lakes located in unaffected areas, revealed again comparable mean background Cu concentration, expressed as total, of 0.43 µg/L (maximum: 3.01 µg/L) (Verta et al., 1990).
Mean background Cu concentrations in the Northern part of Sweden varied between 0.51 µg Cu_{total}/L (range: 0.1-2.0 µg Cu_{total}/L; Borg, 1987) and 0.9 µg Cu_{total}/L (0.25-2.66 µg Cu_{total}/L; Borg, 1983), whereas a median Cu_{total} concentration of 1.0 µg/L (range <0.5 – 2.0 µg/L) was observed in pristine Norwegian lakes (Henriksen and Wright, 1978).
According to Van den Weijden and Middelburg (1989) and Zuurdeeg et al. (1992) it is very difficult to derive background concentrations from fresh surface water in the Netherlands through analytical means because most locations are influenced by anthropogenic inputs. However, Zuurdeeg et al. (1992) could derive background Cu concentrations between 0.8-5.3 µg/L as $Cu_{dissolved}$ and 0.56-2.5 µg/L as total Cu_{total} for Northern Europe.

Figure 1 Copper background concentrations in European surface waters (taken from FOREGS Geochemical Baseline Programme)



For the Dutch situation models were developed and used to derive these background concentrations which can be considered as representative (Van den Hoop, 1995). From these models natural background concentrations for copper between 1.1 and 1.3 µg Cu/L were calculated. The extrapolated background concentration for Cu, expressed as $Cu_{dissolved}$, was 0.44 µg/L for freshwater and 0.25 µg/L for saltwater (Crommentuijn et al., 1997). A background concentration of 1.1 µg Cu_{total}/L was derived for Dutch freshwaters. According to Timmermans et al. (1991), background Cu concentration below detection limit (i.e. <0.3 µg/L) were noticed in Lake Maarsseveen. Other $Cu_{dissolved}$ concentrations reported for the same lake were between 0.3 - 1.8 µg/L (system 1) and 0.4-5.1 µg/L (system 2).

Likewise, background concentrations for German freshwaters cannot easily be estimated from water concentrations. Therefore, an estimation of the background concentration for German surface waters was calculated from the soil concentrations and the particulate-water partitioning coefficient. A mean dissolved background copper concentration of 0.5 µg/L was calculated (Schudoma et al., 1994).

Water samples taken from alpine oligotrophic lakes (Achensee, Drachensee, Mittlerer Plenderlesee, Oberer Plenderlesee en Schwarzsee ob Solden) in the Northern part of

Austria revealed season averaged Cu concentrations between 0.62 and 1.89 µg/L dissolved copper (Kock et al., 1995).
Representative background Cu concentrations for England were measured in the Ivel and Yare rivers from the upper catchment control sites. Background concentrations of respectively 3.5 and 0.5 µg/L total copper were found (Bubb and Lester, 1996). Although it was not very clear how the authors found it, Neal et al. (1996) derived a dissolved background concentration for copper of 1 µg/L.
For Belgium, Richelle et al. (1991) reported copper concentrations in unpolluted pools. The reported total copper concentrations for these pools varied between 0.99 and 1.02 µg/L.
WRc & ECI (2001) reported total and dissolved copper concentrations for 10 European "pristine" natural waters (rivers and lakes) i.e. sites where anthropogenic inputs of metals are insignificant. The investigated river and lake systems were Bihain & Sommerain (Belgium), Lake Clywydog & river Mole (UK), Skarsjön (Sweden), lake Monate and lake Segrino (Italy), Maarkermeer and Ankeveense plassen (Netherlands) and the Rhine at Koblenz (Germany). Copper concentrations for all sites ranged from <0.3 to 3.2 µg Cu_{total}/L and from 0.06 to 3.3 µg $Cu_{dissolved}$/L, respectively.
A summary of the above mentioned background copper concentrations for European surface water is shown in table 4.1.
With these data, a median value of total and dissolved copper background concentration in EU-surface waters derived, i.e. 1.05 and 0.84 µg/L, respectively.
The Zuurdeeg (1992) data for Northern European Lowland were not included in the derivation of a typical Cu-background in European surface waters for 2 reasons:

- Northern Countries were already taken into account (i.e. Finland; Sweden, Norway);
- Reported mean dissolved Cu-concentration was a factor of 2 higher than the total mean concentration, thus making the relevance of these data questionable.

Despite the missing information on quality assurance of the reported data, the typical value of 0.84 µg/L for dissolved copper does confirm the median value of 0.88 µg/L that was generated in the FOREGS Geochemical Mapping Programme.
Background concentrations of copper in groundwater have been reported by various authors. Stuyfzand (1991, 1992) stated that the natural background variation of Cu in groundwater (no anthropogenic input) is situated between 0.1 and 3.2 µg/L. This is in line with the concentration of 1.5 ± 1.5 µg/L that is reported by Meinardi (1999) in groundwater from the Veluwe (The Netherlands). A study by Fraters et al. (2001) revealed that the background concentration of Cu in groundwater depends on the sampling depth and soil type: below 25 m the background is less than 0.63 µg/L, whereas Cu-concentrations in the upper 5 m vary between 12 µg/L (clayey soil) and 25 µg/L (sandy, peaty soil).

Table 4.1 Measured or estimated background copper concentrations in European freshwaters; reported as mean/ median with range (between brackets)

Country	µg Cu/L		Mean/ Median	Reference
	total	dissolved		
Finland	0.28		median	Mannio et al., 1995
	0.43		mean	Verta et al., 1990
	0.11-0.75		range	Ukonmaanoha et al., 1998
Average	**0.39**			
Sweden	0.51 (0.1-2.0)		mean	Borg, 1987
	0.9 (0.25-2.66)		mean	Borg, 1983
	<0.4		1 value	WRc & ECI, 2001
Average:	**<0.63**	**0.3**		
Norway	**1.0** (<0.5-2.0)			Henriksen & Wight, 1978
Northern Europe	1.1 (0.56-2.5)	2.0 (0.8-5.3)	mean	Zuuurdeeg, 2002
The Netherlands	1.1 (0.6-3.0)	0.44	mean	Crommentuijn et al., 1997
	1.33		Mean	Zuurdeeg, 1992
	1.7	0.5	Mean	Van der Weijden & Middelburg, 1989
	3.2	3.3	1 value	WRc & ECI, 2001
Average:	**1.8**	**1.4**		
Germany		0.5	mean	Schudoma et al., 1994
	2.2	1.7		WRc & ECI, 2001
	1.3	0.7	1 value	Van den Berg & Zwolsman, 2000
Average	**1.75**	**0.97**		
Austria		**0.62–1.89**	mean	Kock et al., 1995
England	0.5 – 3.5		mean	Bubb & Lester, 1996
		1	-	Neil et al., 1996
	1.5	1.4	1 value	WRc & ECI, 2001
	2.8	2.2	1 value	WRc & ECI, 2001
Average:	**2.1**	**1.5**		
Belgium	0.99-1.02			Richelle et al., 1991
	<0.3	0.06		WRc & ECI, 2001
	0.9	0.9		WRc & ECI, 2001
Average:	**<0.80**	**0.4**		

Country	µg Cu/L		Mean/ Median	Reference
	total	dissolved		
Italy	<0.4	0.4		WRc & ECI, 2001
	0.5	0.8		WRc & ECI, 2001
Average:	**<0.45**	**0.6**		
Median (+range)	**1.05 (0.39 – 2.1)**	**0. 84 (0.3 – 1.89)**		

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Annexe 5: **Examples of BLM applications**

Rhine-specific HC5-50 values, corrected for bioavailability in accordance to the 2011 EQS guideline, were calculated from 2013 monitoring data.

The ICPR Rhine river database was consulted and all potentially relevant physicochemical data from 2013 (Ca, Mg, Na, K, Cl, SO4, dissolved and total organic carbon content, pH, temperature) were retrieved for the following stations: Rekingen, Weil am Rhein, Lauterbourg/Karlsruhe, Koblenz/Rhine, Koblenz/Mosel, Bimmen, Lobith, Kampen, Maassluis. Site-specific median values (assumption: log-normal distribution) and ranges were derived for each of the parameters and compared to the applicability ranges of the BLM (Table 1).

Table 5.2: Physico-chemistry of the river Rhine samples, collected in 2013 (ICPR website)

Physicochemistry of nine locations along the River Rhine – Median and Min/Max values (mg/L)									
	Bimmen	Kampen	Koblenz		Lauter-burg	Lobith	Maassluis	Rekingen	Weil am Rhein
			Mosel	Rhein					
pH	8.16 (8.0-8.6)	8.05 (7.7-8.4)	7.99 (7.8-8.4)	8.08 (7.9-8.3)	7.97 (7.8-8.2)	8.29 (7.96-8.76)	7.88 (7.1-8.3)	8.18 (8.0-8.3)	8.12 (8.0-8.3)
DOC	No data	3.0 (2.2-4.2)	3.5 (2.4-6.7)	2.2 (1.5-6.1)	1.8 (1.4-2.7)	2.6 (2.02-4.3)	2.9 (2.2-4.3)	2.0 (1.5-4.3)	1.8 (1.4-2.5)
Ca	79 (63-92)	71.4 (60-79)	109.1 (69-370)	64.8 (58-74)	47.7 (22-57)	69.7 (59.9-82.7)	94.2 (63.2-149)	50.6 (33-59)	55.4 (46-68)
Mg	11.6 (10-14)	10.9 (9.1-13.1)	16.7 (12-23)	10.8 (9.5-13)	6.8 (3.3-8.3)	11.0 (9.3-13.3)	84.1 (10.7-312)	9.1 (8.1-10.3)	7.6 (6.4-8.5)
Na	34.3 (21-46)	37.4 (21-53)	46.8 (22-85)	18.8 (13-26)	9.7 (5-14)	37.4 (21.2-50.8)	617.1 (40-2630)	7.1 (5.7-9.4)	8.5 (6.5-11.8)
K	4.2 (3.4-5.1)	4.3 (3.3-5.2)	4.9 (3.9-7.1)	3.2 (2.5-5.7)	1.8 (0.9-2.2)	3.8 (3.1-4.7)	25.6 (3.6-96.8)	1.6 (1.4-1.8)	1.7 (1.4-2.0)
Cl	70.7 (40-99)	65.0 (38-86)	141 (78-229)	31.3 (19-52)	15.9 (11-23)	65.7 (33-93)	1036 (75-4430)	9.9 (7.9-14)	11.9 (8-17)
SO4	54.5 (37-67)	50.8 (32-66)	72 (37-127)	44 (32-53)	22.7 (14-26)	49.4 (33-68)	194.2 (41-650)	28.4 (24-31)	24.5 (20-28)
TOC	3.6 (2.5-6.1)	3.4 (2.4-4.6)	4.3 (2.5-11)	2.5 (1.6-8.0)	2.1 (1.6-4.4)	3.0 (2.1-5.0)	3.3 (2.3-5.7)	2.6 (1.7-5.3)	2.3 (1.6-4.1)

Comparison between Table 5.2 and the copper BLM boundaries indicates that all freshwater samples fall within the BLM boundaries, except for the data from Lobith, with a slightly higher maximum pH value (BLM pH boundary of 8.5; highest measured pH in Rhine of 8.76). The sampling station "Maassluis" represents the estuarine section of the river Rhine and was therefore not further considered

BLM-corrected HC5-50 values were thus determined for all freshwater samples as follows:

- BLM corrected HC5-50 values, determined with the "Cu PNEC estimator V1.3.1" software for each sampling date/station separately.

- pH and DOC are the most critical BLM parameters. If pH and/or DOC values were not available, no calculation was performed. The data from Bimmen could therefore not be used as DOC concentration levels were not reported.
- If data for Ca, Mg or Na were not reported for a specific sample but were available for the station (different sampling periods), then the median value for the site was used as default value (log-normal distribution is assumed: mean = median).
- For the Koblenz, Lauterbourg and Lobith locations, the date of pH-sampling differed from the date that other critical samples were measured. The site-specific average pH was therefore used for these three locations.

The summary statistics of the BLM corrected HC5-50 values, are provided in Table 5.3. For the river Rhine samples assessed, the site-specific median of BLM-corrected values range between 3.6 and 9.8 µg Cu/L, with an overall median of 4.9 µg Cu/L.

Table 5.3: BLM corrected HC5-50 values for river Rhine samples, collected in 2013 (ICPR website)
n= number of samples, Range, P5, P10, P50 = 5th 10th 50th percentiles of the HC5-50 values.

	All data	Kampen	Koblenz		Lauter-burg	Lobith	Rekingen	Weil am Rhein
			Mosel	Rhein				
N	169	13	26	26	26	26	26	26
Range	2.5-21.7	5.0-16.1	7.0-21.7	3.4-16.0	3.7-7.6	4.5-9.0	2.5-7.7	2.5-6.4
P5	2.7	5.1	8.0	3.6	3.8	4.6	2.5	2.5
P10	3.1	5.7	8.1	3.8	4.0	4.7	2.6	2.6
P50	4.9	9.3	9.8	4.8	4.6	5.3	3.6	3.6

Conclusion and applications

Chronic BLM calculations, applied to the 2013 freshwater River Rhine monitoring data, resulted in HC5-50 values ranging between 2.5 and 22 µg Cu/L. Site-specific median BLM-corrected HC5-50 values, relevant to the yearly average EQS derivations, range between 3.6 and 9.8 µg Cu/L.

Following the 2011 EQS guideline, these HC5-50 values can be used as a basis for deriving (1) site-specific yearly average EQS values or (2) site specific bio-availability factors (BIOF= EQS-Rhine generic/ EQS site) and site specific bio-availability corresponding bio-available copper concentrations (µg bio-available Cu/L= (µg dissolved copper/L)/ BIOF.